

# Lead, Genetic Susceptibility, and Risk of Adult Brain Tumors

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## Abstract

**Background:** Although few etiologic factors for brain tumors have been identified, limited data suggest that lead may increase the risk of brain tumors, particularly meningioma. The *ALAD G177C* polymorphism affects the toxicokinetics of lead and may confer genetic susceptibility to adverse effects of lead exposure.

**Methods:** We examined occupational exposure to lead and risk of brain tumors in a multisite, hospital-based, case-control study of 489 patients with glioma, 197 with meningioma, and 799 non-cancer controls frequency matched on hospital, age, sex, race/ethnicity, and residential proximity to hospital. *ALAD* genotype was assessed by a Taqman assay for 355 glioma patients, 151 meningioma patients, and 505 controls. Exposure to lead was estimated using a rigorous questionnaire-based exposure assessment strategy incorporating lead measurement and other occupational data abstracted from published articles and reports.

**Results:** Increased risk of meningioma with occupational lead exposure (estimated by odds ratios and 95% confidence intervals) was most apparent in individuals with the *ALAD2* variant allele, for whom risk increased from 1.1 (0.3-4.5) to 5.6 (0.7-45.5) and 12.8 (1.4-120.8) for estimated cumulative lead exposures of 1 to 49  $\mu\text{g}/\text{m}^3\text{-y}$ , 50 to 99  $\mu\text{g}/\text{m}^3\text{-y}$ , and  $\geq 100 \mu\text{g}/\text{m}^3\text{-y}$ , respectively, compared with unexposed individuals (two-sided  $P$  trend = 0.06). This relationship became stronger after excluding occupational lead exposures characterized by a low confidence level or occurring in the 10 years before meningioma diagnosis. Occupational lead exposure was not associated with glioma risk.

**Conclusions:** Although our results indicate that lead may be implicated in meningioma risk in genetically susceptible individuals, these results need to be interpreted with caution given the small numbers of exposed cases with a variant genotype. (Cancer Epidemiol Biomarkers Prev 2006;15(12):2514-20)

## Introduction

The toxicity of lead has been known for centuries, with well-documented adverse effects on the hematopoietic, gastrointestinal, urinary, cardiovascular, and nervous systems (1). Based on sufficient evidence of animal carcinogenicity and limited evidence of human carcinogenicity, inorganic lead was recently reclassified from a "possible" to a "probable" human carcinogen by the International Agency Research on Cancer (IARC) (2).

Whereas some epidemiologic studies have described increased risk of brain tumors with potential lead exposure (3-6), particularly for meningioma (7-9), other evaluations report no significant association between lead and brain cancer (10-12). The inconsistency of previous reports may be due to small numbers of brain tumor cases, limited exposure assessment for lead, or lack of consideration of brain tumor type.

Possible genetic susceptibility to the potential relationship between lead and brain tumor risk has not been previously evaluated. The enzyme  $\delta$ -aminolevulinic acid dehydratase (*ALAD*), which catalyzes the second step of heme synthesis and is coded by the *ALAD* gene, is strongly inhibited by lead. The most commonly studied polymorphism in the gene, *ALAD G177C* (dbSNP ID: rs1800435), contains a G to C transversion at position 177 of the coding region and has two codominant

alleles, *ALAD1* and *ALAD2*, with an *ALAD2* allele frequency ranging from 6% to 20% in Caucasian populations, 3% to 11% in Asian populations, and approximately 3% in African-American populations (13).

Although some studies indicate no significant difference in blood lead levels between *ALAD1* and *ALAD2* genotypes (14-20), individuals with the *ALAD2* allele are generally reported to have higher mean blood lead levels than *ALAD1* homozygotes at higher levels of exposure to lead (21-26), possibly due to increased binding of lead to the *ALAD2* allele (27). How *ALAD* genotype influences the distribution of lead to target organs, however, is still unknown. One competing possibility is that increased binding of lead to *ALAD2* could result in lower lead levels in other tissues, decreasing the risk of adverse health effects. Alternatively, increased blood lead levels with the *ALAD2* allele could result in a higher dose delivered to other organs, thus increasing the risk of adverse health effects. Previous studies have indicated that *ALAD2* might be protective against the neurotoxic/neurobehavioral effects of lead (28, 29). However, it has also been observed that *ALAD2* might confer increased risk of amyotrophic lateral sclerosis (19) and meningioma (30).

The availability of detailed exposure assessment for lead and blood samples for genotyping allowed us to evaluate the role of job-related lead exposure and risk of adult glioma and meningioma according to *ALAD G177C* genotype in a large case-control study of brain tumors.

## Materials and Methods

**Study Setting and Population.** A detailed description of study methods can be found elsewhere (31). Briefly, subjects for a case-control study of brain tumors were enrolled between

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1994 and 1998 from three hospitals that are regional referral centers for brain tumors, located in Phoenix (Arizona), Boston (Massachusetts), and Pittsburgh (Pennsylvania). The study protocol was approved by the Institutional Review Board of each participating institution, and written informed consent was obtained from each patient or proxy.

Eligible patients were ages  $\geq 18$  years with a first intracranial glioma, meningioma [International Classification of Diseases for Oncology (second edition) codes 9530-9538], or acoustic neuroma [International Classification of Diseases for Oncology (second edition) codes 9560] diagnosed during or within the 8 weeks preceding hospitalization. Ninety-two percent of eligible brain tumor patients agreed to participate. Acoustic neuroma patients will not be described further, since our analysis focuses on glioma and meningioma. Four hundred and eighty-nine patients with glioma and 197 patients with meningioma were enrolled. All diagnoses were confirmed by microscopy.

Study controls were patients admitted to the same hospitals as cases for a variety of non-neoplastic conditions, including injuries (25%), circulatory system disorders (22%), musculoskeletal disorders (22%), and digestive disorders (12%). Controls were frequency matched in a 1:1 ratio to all brain tumor patients based on age (in years) at interview (18-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80-99), race/ethnicity (non-Hispanic white, Hispanic, African-American, other), sex (male, female), hospital (Phoenix, Boston, Pittsburgh), and residential proximity to the hospital in miles (0-4, 5-14, 15-29, 30-49,  $\geq 50$ ). Seven hundred and ninety-nine patients, representing 86% of all contacted controls, were enrolled.

Blood samples were collected from 382 (78%) patients with glioma, 158 (80%) with meningioma, and 540 (68%) controls. Refusal to provide a blood sample was higher for controls (24%) than for cases (14%).

**Data Collection.** A trained research nurse administered a structured in-person interview to each patient or proxy (if the subject was functionally impaired, too ill to respond, or deceased). Interviews were conducted by proxy for 78 (16%) glioma cases, 15 (8%) meningioma cases, and 23 (3%) controls. For all patients, a lifetime occupational history, including every job held for at least 6 months after 16 years of age, was collected, along with information on other known or potential risk factors for brain tumors, such as previous medical exposures or conditions, selected dietary exposures, and use of cellular telephones. Job-specific questions developed by an expert industrial hygienist were asked for jobs with expected exposure to specific chemical and physical agents to assess the probability, frequency, and concentration of those agents (32). Interviews were administered in the hospital and generally lasted 1.5 h. Follow-up occupational questions were asked in a subsequent brief interview within 6 weeks of initial patient interview. Eighty percent of cases were interviewed within 3 weeks of diagnosis, and all cases were interviewed within 8 weeks. The vast majority of controls (90%) were interviewed within a year of symptom onset, and all were interviewed within 5 years of symptom onset.

**Exposure Assessment for Lead.** A comprehensive quantitative lead exposure database was created before assessment of exposures. Estimates derived from this database were assigned to work history information from patient interviews.

**Lead Database.** A lead exposure database was created by abstracting standardized information, including uses of lead, measurements of lead in air and blood, and probability and frequency of lead exposure from over 475 peer-reviewed articles and industrial hygiene technical reports. These data were compiled and summarized by job and decade to create matrices of probability, concentration, and frequency of lead exposure. Probability of lead exposure indicated likelihood of

lead exposure (0%, 1-9%, 10-49%, 50-89%, or  $\geq 90\%$ ); concentration referred to the estimated concentration of airborne lead to which the subject was exposed (0, 5-9, 10-29, 30-49, 50-249, or  $\geq 250 \mu\text{g}/\text{m}^3$ ), and frequency estimated the average proportion of an individual's time during which he or she was likely to be exposed to lead (<1, 1-9, 10-29, or 30-40 h/week). Where information was lacking (<5% of jobs), estimates were developed by an expert industrial hygienist, with the occasional use of a comprehensive job-exposure matrix developed for a Canadian study (33).

**Exposure Assessment for Study Patients.** The 1,581 study patients held a total of 8,535 jobs. Job information on patients was stripped of subject identifiers, including case status. Standardized job and industry codes, used in related articles from the brain tumor study (34, 35), were not used here. Rather, in this study, estimates of lead exposure probability, concentration, and frequency were derived from the lead database matrices (described above) based on the actual job title reported and modified according to information provided by the subject on tasks done, personal protective equipment worn, industry, and other individual-specific information. The estimates were assigned to each job for each subject. Finally, each job-specific estimate of lead level was assigned a confidence of low, medium, or high to reflect the quality of data on which the estimate was based. All information was reviewed by an experienced industrial hygienist.

**Processing of Blood Samples.** DNA was extracted from blood samples using a phenol-chloroform method (36), and *ALAD* genotyping was conducted by the Core Genotyping Facility of the National Cancer Institute (NCI) using a medium-throughput Taqman assay (30). Quality control samples revealed a 98% agreement for *ALAD* call between three nonstudy replicates and a 90% concordance rate for study duplicates (study samples compared with masked relabels). *ALAD* genotyping was successfully conducted for 94% of samples.

**Statistical Analyses.** Statistically significant departure from Hardy-Weinberg equilibrium for controls was assessed using the  $\chi^2$  test. Unconditional logistic regression was used to calculate adjusted odds ratios (OR) and 95% confidence intervals (95% CI). Models were run including all study matching factors (age, sex, race/ethnicity, hospital, and residential proximity to the hospital), and including age and sex only. Matching factors were entered as indicator variables.

For all exposure metrics, patients were considered lead exposed if they had ever worked in a job with lead exposure probability of  $\geq 10\%$ . Estimated lead exposure was examined in several ways: ever exposed at a concentration  $\geq 10 \mu\text{g}/\text{m}^3$ , years of exposure ( $y$ ), highest intensity exposure ( $\mu\text{g}/\text{m}^3$ ), lifetime cumulative exposure ( $\mu\text{g}/\text{m}^3\text{-y}$ ), and average lifetime intensity of exposure while exposed ( $\mu\text{g}/\text{m}^3$ ). Lifetime cumulative lead exposure was calculated by multiplying the number of years in each lead-exposed job over a participant's lifetime by category midpoints of concentration and frequency. Average exposure over the participant's life was derived by dividing lifetime cumulative exposure by number of years exposed to lead.

Analyses were repeated excluding low-confidence lead exposure estimates and also lead exposure occurring 5 and 10 years before diagnosis (to account for lag between time of exposure and tumor formation). To test for the influence of control group composition on the results, the models were run excluding each major category of control diagnosis at a time. Proxy interviews were excluded from models to test whether interview quality may have affected our results, and possible confounding by education was examined by including this variable in analyses. Finally, a separate analysis was conducted for glioblastoma [International Classification of Diseases for Oncology (second edition) codes 9440, 9441, and 9442].

Effect modification of the relationship between lead and each brain tumor type was assessed by stratifying models according to *ALAD* genotype. Because very few patients were *ALAD2-2* homozygotes, we compared the combined category of *ALAD1-2* heterozygotes and *ALAD2-2* homozygotes with the referent category of *ALAD1-1* homozygotes.

## Results

The final study population of 1,485 patients included 1,011 patients with genotyping results and 474 without genotyping results (Table 1). Tumor cases who were male and those in the oldest age bracket were less likely to be genotyped. Also less likely to be genotyped were glioma patients who were less educated and those treated at Phoenix. Genotyped meningioma patients had a slightly higher proportion of participants with less than high school education than did meningioma patients without genotyping. Control patients with and without genotyping were generally comparable. Overall, patients with tumors tended to be older and more educated than controls. Thirty-six percent of patients were exposed to some occupational lead in their lifetime, with common lead-exposed occupations including military jobs, law enforcement officers, construction workers, drivers, mechanics, garage attendants, and painters.

Risk of glioma was not associated with any lead exposure metric in overall or sex-specific analyses (Table 2; results not shown for sex-specific analyses). Cumulative lead exposure was weakly associated with meningioma when all individuals were included, with an estimated 14% increase in risk for a 50  $\mu\text{g}/\text{m}^3\text{-y}$  increase in cumulative lead exposure. This pattern was seen more clearly in males (OR, 1.2; 95% CI, 1.01-1.4) than in females (OR, 0.6; 95% CI, 0.3-1.2). A similar gender difference was observed for participants ever exposed to lead (OR, 1.6; 95% CI, 0.7-3.5 for males versus OR, 0.4; 95% CI,

0.2-0.9 for females). Although no overall association with meningioma was seen for metrics other than cumulative lead exposure, a clear and consistent pattern of increased risk of meningioma in individuals with the *ALAD2* allele was observed with all measures of lead exposure. Compared with unexposed individuals, the risk of meningioma (estimated by OR and 95% CI) among *ALAD2* patients who ever had a job with lead exposure was 2.4 (0.7-8.8; Table 2). Meningioma risk increased with duration of exposure to lead ( $P$  trend = 0.09), lifetime cumulative exposure to lead ( $P$  trend = 0.06), lifetime average exposure to lead ( $P$  trend = 0.02; Table 3), and highest exposure to lead. These results became stronger after exclusion of jobs with low confidence exposure (Table 3) or exclusion of lead exposures occurring within 10 years of brain tumor diagnosis (Table 4). When lead exposures occurring within 10 years of meningioma diagnosis were excluded, risk estimates in individuals with the *ALAD2* variant were 1.4 (0.3-6.2), 10.3 (1.1-95.9), and 16.3 (1.6-162.3) for lifetime cumulative lead exposures of 1 to 49  $\mu\text{g}/\text{m}^3\text{-y}$ , 50 to 99  $\mu\text{g}/\text{m}^3\text{-y}$ , and  $\geq 100$   $\mu\text{g}/\text{m}^3\text{-y}$  of lead, respectively.

In the absence of lead exposure, individuals with the *ALAD2* genotype were not at increased risk of meningioma (Table 5). When lead was present, however, the risk associated with the *ALAD2* allele increased sharply with rising average annual or cumulative lifetime exposure to lead.

We examined for potential bias that may have occurred due to use of hospital controls by systematically excluding control subgroups from our analysis; ORs did not change appreciably. Results were also very similar when controlled for education. Exclusion of proxy interviews (Table 4) and models including all matching factors for categorical variables, also yielded very similar results to those reported in Tables 2 and 3, with risk estimates for meningioma being higher but less precise (data not shown for models with all matching factors). A separate analysis of glioblastoma yielded results similar to those for all gliomas combined (Table 4).

**Table 1. Demographic characteristics for individuals with glioma and meningioma and frequency-matched controls in the NCI Adult Brain Tumor Study, 1994-1998**

| Characteristic                           | Genotyped samples, <i>n</i> (%) |                              |                              | No genotyping data available, <i>n</i> (%)* |                             |                            |
|--|---------------------------------|------------------------------|------------------------------|---|-----------------------------|----------------------------|
|  | Glioma ( <i>n</i> = 355)        | Meningioma ( <i>n</i> = 151) | Controls † ( <i>n</i> = 505) | Glioma ( <i>n</i> = 134)                    | Meningioma ( <i>n</i> = 46) | Controls ( <i>n</i> = 294) |
| Sex                                      |                                 |                              |                              |   |                             |                            |
| Male                                     | 192 (54.1)                      | 32 (21.2)                    | 234 (46.3)                   | 85 (63.4)                                   | 14 (30.4)                   | 129 (43.9)                 |
| Female                                   | 163 (45.9)                      | 119 (78.8)                   | 271 (53.7)                   | 49 (36.6)                                   | 32 (69.6)                   | 165 (56.1)                 |
| Race/ethnicity                           |                                 |                              |                              |   |                             |                            |
| White, non-Hispanic                      | 323 (91.0)                      | 123 (81.5)                   | 450 (89.1)                   | 121 (90.3)                                  | 40 (87.0)                   | 265 (90.1)                 |
| Hispanic                                 | 19 (5.4)                        | 12 (8.0)                     | 36 (7.1)                     | 7 (5.2)                                     | 2 (4.4)                     | 18 (6.1)                   |
| Black                                    | 7 (2.0)                         | 9 (6.0)                      | 11 (2.2)                     | 3 (2.2)                                     | 0 (0.0)                     | 8 (2.7)                    |
| Other                                    | 6 (1.7)                         | 7 (4.6)                      | 8 (1.6)                      | 3 (2.2)                                     | 4 (8.7)                     | 3 (1.0)                    |
| Age at interview (y)                     |                                 |                              |                              |   |                             |                            |
| 18-29                                    | 41 (11.6)                       | 2 (1.3)                      | 69 (13.7)                    | 17 (12.7)                                   | 2 (4.4)                     | 32 (10.9)                  |
| 30-49                                    | 131 (36.9)                      | 55 (36.4)                    | 202 (40.0)                   | 44 (32.8)                                   | 16 (34.8)                   | 109 (37.1)                 |
| 50-69                                    | 121 (34.1)                      | 68 (45.0)                    | 163 (32.3)                   | 45 (33.6)                                   | 17 (37.0)                   | 113 (38.4)                 |
| 70-90                                    | 62 (17.5)                       | 26 (17.2)                    | 71 (14.1)                    | 28 (20.9)                                   | 11 (23.9)                   | 40 (13.6)                  |
| Educational level‡                       |                                 |                              |                              |   |                             |                            |
| <High school                             | 38 (11.1)                       | 20 (13.3)                    | 69 (13.9)                    | 26 (19.9)                                   | 4 (8.7)                     | 36 (12.7)                  |
| High school or GED                       | 92 (26.9)                       | 47 (31.3)                    | 144 (29.1)                   | 30 (22.9)                                   | 10 (21.7)                   | 90 (31.8)                  |
| 1- to 3-year college or technical school | 94 (27.5)                       | 49 (32.7)                    | 164 (33.1)                   | 36 (27.5)                                   | 19 (41.3)                   | 81 (28.6)                  |
| 4-year college                           | 62 (18.1)                       | 16 (10.7)                    | 59 (11.9)                    | 27 (20.6)                                   | 7 (15.2)                    | 46 (16.3)                  |
| Graduate school                          | 56 (16.4)                       | 18 (12.0)                    | 59 (11.9)                    | 12 (9.2)                                    | 6 (13.0)                    | 30 (10.6)                  |
| Unknown                                  | 13                              | 1                            | 10                           | 3   | 0                           | 11                         |
| Hospital site                            |                                 |                              |                              |   |                             |                            |
| Phoenix, AZ                              | 162 (45.6)                      | 75 (49.7)                    | 258 (51.1)                   | 82 (61.2)                                   | 24 (52.2)                   | 147 (50.0)                 |
| Boston, MA                               | 126 (35.5)                      | 62 (41.1)                    | 164 (32.5)                   | 27 (20.2)                                   | 17 (37.0)                   | 56 (19.1)                  |
| Pittsburgh, PA                           | 67 (18.9)                       | 14 (9.3)                     | 83 (16.4)                    | 25 (18.7)                                   | 5 (10.9)                    | 91 (31.0)                  |

\*Individuals did not submit blood, samples did not pass quality control for genotyping, or no call was obtained.

†Controls were matched to the total case group, including glioma, meningioma, and acoustic neuroma.

‡Percentage based on nonmissing values.

**Table 2. Estimated risk of meningioma and glioma with exposure to lead by *ALAD G177C* genotype in the NCI Brain Tumor Study, 1994-1998**

|  | Cases/<br>controls (n) | Overall,<br>OR (95% CI) | Cases/<br>controls (n) | Genotyped<br>individuals,<br>OR (95% CI) | Cases/<br>controls (n) | <i>ALAD1</i><br>individuals,<br>OR (95% CI) | Cases/<br>controls (n) | <i>ALAD2</i><br>individuals,<br>OR (95% CI)* |
|--|------------------------|-------------------------|------------------------|--|------------------------|---|------------------------|--|
| <b>Meningioma</b>  |                        |                         |                        |  |                        |   |                        |  |
| Ever exposed to lead <sup>†</sup>  |                        |                         |                        |  |                        |   |                        |  |
| No   | 151/510                | 1.0 (reference)         | 117/311                | 1.0 (reference)                          | 95/254                 | 1.0 (reference)                             | 22/57                  | 1.0 (reference)                              |
| Yes  | 46/287                 | 0.8 (0.5-1.3)           | 34/194                 | 0.7 (0.4-1.3)                            | 21/166                 | 0.5 (0.3-1.0)                               | 13/28                  | 2.4 (0.7-8.8)                                |
|  |                        |                         |                        |  |                        |   |                        | <i>P</i> interaction = 0.01                  |
| Years exposed to lead <sup>‡</sup>   |                        |                         |                        |  |                        |   |                        |  |
| <5   | 170/627                | 1.0 (reference)         | 129/392                | 1.0 (reference)                          | 103/324                | 1.0 (reference)                             | 26/68                  | 1.0 (reference)                              |
| 5-14   | 12/94                  | 0.8 (0.4-1.5)           | 10/59                  | 0.9 (0.4-1.9)                            | 7/49                   | 0.8 (0.3-2.0)                               | 3/10                   | 1.4 (0.3-7.3) <sup>§</sup>                   |
| ≥15  | 15/76                  | 1.2 (0.6-2.2)           | 12/54                  | 1.2 (0.5-2.5)                            | 6/47                   | 0.7 (0.3-1.8)                               | 6/7                    | 4.6 (0.9-22.8)                               |
| $\beta$ , <i>P</i> <sup>  </sup>   |                        | 0.009, 0.5              |                        | 0.005, 0.7                               |                        | -0.01, 0.5                                  |                        | 0.05, 0.09                                   |
| OR for a 10-year increase in<br>years exposed to lead                                    |                        | 1.09                    |                        | 1.05                                     |                        | 0.87  |                        | 1.67   |
|  |                        |                         |                        |  |                        |   |                        | <i>P</i> interaction = 0.03                  |
| Cumulative exposure to lead ( $\mu\text{g}/\text{m}^3\text{-y}$ )                        |                        |                         |                        |  |                        |   |                        |  |
| 0  | 141/476                | 1.0 (reference)         | 110/290                | 1.0 (reference)                          | 88/238                 | 1.0 (reference)                             | 22/52                  | 1.0 (reference)                              |
| 1-49   | 34/183                 | 1.0 (0.6-1.5)           | 23/122                 | 0.8 (0.5-1.5)                            | 19/103                 | 0.9 (0.5-1.6)                               | 4/19                   | 1.1 (0.3-4.5) <sup>§</sup>                   |
| 50-99  | 8/64                   | 0.8 (0.3-1.8)           | 7/45                   | 0.8 (0.3-2.2)                            | 3/35                   | <b>0.4 (0.1-1.6)</b>                        | 4/10                   | 5.6 (0.7-45.5) <sup>§</sup>                  |
| ≥100   | 14/74                  | 1.1 (0.5-2.2)           | 11/48                  | 1.1 (0.5-2.6)                            | 6/44                   | 0.7 (0.2-1.8)                               | 5/4                    | 12.8 (1.4-120.8) <sup>§</sup>                |
| $\beta$ , <i>P</i>   |                        | 0.003, 0.06             |                        | 0.003, 0.1                               |                        | 0.001, 0.8                                  |                        | 0.007, 0.06                                  |
| OR for a 50 $\mu\text{g}/\text{m}^3\text{-y}$ increase in<br>cumulative exposure to lead |                        | 1.14                    |                        | 1.15                                     |                        | 1.04  |                        | 1.39   |
|  |                        |                         |                        |  |                        |   |                        | <i>P</i> interaction = 0.08                  |
| <b>Glioma</b>  |                        |                         |                        |  |                        |   |                        |  |
| Ever exposed to lead   |                        |                         |                        |  |                        |   |                        |  |
| No   | 294/510                | 1.0 (reference)         | 213/311                | 1.0 (reference)                          | 184/254                | 1.0 (reference)                             | 29/57                  | 1.0 (reference)                              |
| Yes  | 190/287                | 0.8 (0.6-1.1)           | 140/194                | 0.8 (0.5-1.1)                            | 115/166                | 0.7 (0.5-1.1)                               | 25/28                  | 0.7 (0.2-1.8)                                |
|  |                        |                         |                        |  |                        |   |                        | <i>P</i> interaction = 0.1                   |
| Years exposed to lead  |                        |                         |                        |  |                        |   |                        |  |
| <5   | 378/627                | 1.0 (reference)         | 276/392                | 1.0 (reference)                          | 236/324                | 1.0 (reference)                             | 40/68                  | 1.0 (reference)                              |
| 5-14   | 40/94                  | 0.5 (0.4-0.8)           | 32/59                  | 0.6 (0.4-1.0)                            | 29/49                  | 0.7 (0.4-1.1)                               | 3/10                   | 0.3 (0.1-1.5) <sup>§</sup>                   |
| ≥15  | 66/76                  | 1.0 (0.7-1.5)           | 45/54                  | 0.9 (0.6-1.4)                            | 34/47                  | 0.8 (0.5-1.3)                               | 11/7                   | 1.6 (0.5-5.2)                                |
| $\beta$ , <i>P</i>   |                        | 0.004, 0.5              |                        | -0.002, 0.8                              |                        | -0.007, 0.5                                 |                        | 0.02, 0.5                                    |
| OR for a 10-year increase in<br>years exposed to lead                                    |                        | 1.04                    |                        | 0.98                                     |                        | 0.94  |                        | 1.19   |
|  |                        |                         |                        |  |                        |   |                        | <i>P</i> interaction = 0.5                   |
| Cumulative exposure to lead ( $\mu\text{g}/\text{m}^3\text{-y}$ )                        |                        |                         |                        |  |                        |   |                        |  |
| 0  | 276/476                | 1.0 (reference)         | 202/290                | 1.0 (reference)                          | 173/238                | 1.0 (reference)                             | 29/52                  | 1.0 (reference)                              |
| 1-49   | 115/183                | 0.8 (0.6-1.1)           | 88/122                 | 0.8 (0.5-1.1)                            | 75/103                 | 0.8 (0.5-1.2)                               | 13/19                  | 0.8 (0.3-2.1)                                |
| 50-99  | 32/64                  | 0.6 (0.4-1.0)           | 20/45                  | 0.6 (0.2-0.8)                            | 18/35                  | 0.5 (0.3-1.0)                               | 2/10                   | 0.2 (0.0-1.0) <sup>§</sup>                   |
| ≥100   | 61/74                  | 0.9 (0.6-1.4)           | 43/48                  | 0.8 (0.5-1.4)                            | 33/44                  | 0.7 (0.4-1.3)                               | 10/4                   | 2.1 (0.5-9.3) <sup>§</sup>                   |
| $\beta$ , <i>P</i>   |                        | 0.0, 1.0                |                        | 0.0, 0.7                                 |                        | -0.001, 0.6                                 |                        | 0.001, 0.7                                   |
| OR for a 50 $\mu\text{g}/\text{m}^3\text{-y}$ increase in<br>cumulative exposure to lead |                        | 1.00                    |                        | 0.98                                     |                        | 0.97  |                        | 1.06   |
|  |                        |                         |                        |  |                        |   |                        | <i>P</i> interaction = 0.2                   |

\*Includes *ALAD1-2* heterozygotes and *ALAD2-2* homozygotes.<sup>†</sup>Models for ever being exposed to lead and models for underlying continuous variables were adjusted for matching factors: age, sex, race, hospital, and distance of residence from hospital.<sup>‡</sup>Models using exposure categories were adjusted for age and sex only.<sup>§</sup>Number of exposed cases or controls is less than five.<sup>||</sup>Likelihood ratio test for interaction between lead and *ALAD* genotype based on underlying continuous variable.

## Discussion

Overall, we found weak evidence of an association between cumulative lead exposure and risk of meningioma, with this pattern being seen only in males. However, risk of meningioma was consistently increased with all lead exposure metrics for individuals carrying the *ALAD2* allele. Previous studies with more rudimentary exposure information have reported an overall increase in meningioma risk with lead exposure (3, 7-9). Although these studies did not examine possible susceptibility by *ALAD* genotype, we would expect that if their study populations had only a small proportion of high-risk individuals, lesser magnitudes of overall risk would have been reported than we observed for patients with the *ALAD2* allele. Overall meningioma risk estimates of 1.9 and 2.4 were observed for lead-exposed individuals in the larger of those studies, comparable with the 2.4-fold excess we observed for lead-exposed individuals with the *ALAD2* variant but considerably lower than magnitudes of risk we observed for highly

lead-exposed *ALAD2* individuals. This might indicate lower levels of lead exposure in our population compared with previous study populations. Lower exposure levels may also explain our weak overall observation of meningioma risk with lead exposure. We observed no association between lead exposure and risk of glioma.

An earlier analysis of our data showed elevated risk of meningioma among individuals with the *ALAD2* variant, with the risk being stronger in males than in females (30). The newly available exposure assessment for lead revealed that neither lead nor the variant genotype had a strong effect on meningioma risk in the absence of the other, indicating that increased risk was most likely to occur when both lead and the variant genotype are present. Men with meningioma were more likely to have been highly exposed to lead despite the higher incidence of meningioma in women. Higher levels of lead exposure in men might explain our observation that increased overall risk of meningioma with lead exposure was seen more clearly in men than in women.

Our study had a large number of histologically confirmed brain tumors, high participation rates, rapid interviewing of subjects, detailed individual exposure assessment for lead, and availability of blood samples for genotyping. Previous studies of lead and brain tumor have generally faced a trade-off between number of brain tumor cases (range, 3-27,060) and quality of lead exposure information, assessed by methods as diverse as membership in a lead-exposed occupational cohort (37-41), use of job exposure matrices applied to a one-time assessment of occupation (4, 5, 7, 9, 11), or blood lead levels (6), which are an inadequate measure of long-term lead exposure.

Brain tumors are a rare outcome, and thus, the sample size of lead-exposed individuals with the variant genotype in this study was small. Our statistical power to detect gene-environment interaction was therefore quite low, and estimates of risk were quite imprecise as reflected by the large confidence intervals. Although we observed strong associations between lead and meningioma in individuals with the *ALAD2* variant, these findings may reflect the play of chance and need to be replicated in a larger study. Another limitation of our study was the lack of biological measurements of cumulative lead exposure, which would have reduced misclassification and incorporated both occupational and environmental sources of exposure. However, because exposure in this study was assessed without knowledge of diagnosis, misclassification is likely to have been nondifferential. If a weak association for glioma existed, it is possible that exposure misclassification prevented us from detecting this. Future studies of the relationship between lead and brain tumor risk should consider collection of a validated and relatively noninvasive long-term biomarker of lead exposure, such as bone lead (42).

Results of a hospital-based case-control study can be biased if the exposure under study is associated with conditions leading to enrollment in the control series (43). However, excluding one major control subgroup at a time did not appreciably change

results. Our observed relationships are also unlikely to be explained by correlation of lead with another agent because it is unlikely that the same agent would be present in all lead-exposed occupations and explain the observed dose-response relationship with lead. Lead-exposed meningioma patients with the *ALAD2* allele held a variety of job titles, including driver, printer, plastic manufacturing worker, automobile mechanic, copper worker, and rifle instructor.

Lead strongly inhibits the enzyme ALAD, which causes the heme precursor ALA to accumulate. Given that inorganic lead is a suspected carcinogen (2) and that ALA is mutagenic (44, 45) and can cause oxidative stress (45-48), increased risk of meningioma for lead-exposed individuals with the *ALAD2* genotype could be driven either by lead or by ALA accumulation. However, reported differences in urinary or plasma levels of ALA by genotype have been inconsistent (18, 20, 49). On the other hand, individuals with the *ALAD2* allele are generally reported to have higher blood lead levels than individuals with the *ALAD1* allele at higher levels of exposure to lead (21-26), raising the possibility that more lead is delivered to the brain in individuals with the *ALAD2* allele.

Evidence to date suggests that lead might act through one or more facilitative mechanisms that increase the carcinogenicity of other known carcinogens (50, 51). *In vitro* gene-tox assays for lead, studies using human cells, and genotoxic studies of lead in nonhuman species have generally shown minor or absent genotoxic effects, whereas most studies of lead-exposed human populations have reported chromosomal toxicity, including increased chromatid gaps, breaks, and exchanges, and a higher frequency of chromosomal aberrations even at relatively low blood levels. Lead has also been shown to interfere with repair of DNA damage induced by agents of known genotoxicity (52, 53) and increase the *in vitro* mutagenicity of known carcinogens (54). It is thus possible that exposures co-occurring with lead (e.g., solvents) contributed to the increased risk of meningioma in our study.

**Table 3. Estimated risk of meningioma with categories of lead exposure by *ALAD G177C* genotype in the NCI Brain Tumor Study, 1994-1998**

| Meningioma   | Genotyped individuals |                 | <i>ALAD1</i> individuals |                      | <i>ALAD2</i> individuals* |                               |
|--|-----------------------|-----------------|--------------------------|----------------------|---------------------------|-------------------------------|
|  | Cases/controls (n)    | OR (95% CI)     | Cases/controls (n)       | OR (95% CI)          | Cases/controls (n)        | OR (95% CI)                   |
| Cumulative exposure to lead, medium to high confidence exposures only ( $\mu\text{g}/\text{m}^3\text{-y}$ ) <sup>†</sup> |                       |                 |                          |                      |                           |                               |
| 0  | 111/298               | 1.0 (reference) | 89/244                   | 1.0 (reference)      | 22/53                     | 1.0 (reference)               |
| 1-49   | 17/104                | 0.9 (0.5-1.7)   | 14/89                    | 0.9 (0.4-1.8)        | 4/19                      | 1.2 (0.2-6.2) <sup>‡</sup>    |
| 50-99  | 9/34                  | 1.8 (0.7-4.7)   | 5/25                     | 1.3 (0.4-4.1)        | 4/5                       | 6.7 (0.8-55.2) <sup>‡</sup>   |
| $\geq 100$   | 8/39                  | 1.2 (0.5-3.1)   | 4/36                     | 0.6 (0.2-2.1)        | 5/3                       | 18.8 (1.6-221.5) <sup>‡</sup> |
| $\beta$ , $P$ <sup>§</sup>   |                       | 0.003, 0.1      |                          | 0.001, 0.6           |                           | 0.006, 0.1                    |
| OR for a 50 $\mu\text{g}/\text{m}^3\text{-y}$ increase in cumulative exposure to lead                                    |                       | 1.16            |                          | 1.07                 |                           | 1.34                          |
| Average exposure to lead ( $\mu\text{g}/\text{m}^3$ )  |                       |                 |                          |                      |                           |                               |
| 0  | 110/290               | 1.0 (reference) | 88/238                   | 1.0 (reference)      | 22/52                     | 1.0 (reference)               |
| 1-4  | 19/102                | 0.8 (0.4-1.5)   | 16/81                    | 0.9 (0.5-1.8)        | 3/21                      | 0.7 (0.1-3.3) <sup>‡</sup>    |
| 5-9  | 15/93                 | 0.9 (0.4-1.7)   | 9/84                     | 0.5 (0.2-1.3)        | 6/9                       | 9.4 (1.3-67.9)                |
| $\geq 10$  | 7/20                  | 1.7 (0.6-4.7)   | 3/17                     | <b>0.9 (0.2-3.4)</b> | 4/3                       | 11.3 (1.1-113.9) <sup>‡</sup> |
| $\beta$ , $P$  |                       | 0.02, 0.5       |                          | -0.04, 0.4           |                           | 0.23, 0.02                    |
| OR for a 5 $\mu\text{g}/\text{m}^3$ increase in average exposure to lead   |                       | 1.12            |                          | 0.82                 |                           | 3.12                          |
| Highest exposure to lead ( $\mu\text{g}/\text{m}^3$ ) <sup>  </sup>  |                       |                 |                          |                      |                           |                               |
| <10  | 117/311               | 1.0 (reference) | 95/254                   | 1.0 (reference)      | 22/57                     | 1.0 (reference)               |
| 10-29  | 12/68                 | 0.6 (0.3-1.3)   | 7/57                     | 0.4 (0.2-1.1)        | 5/11                      | 1.8 (0.4-7.8) <sup>‡</sup>    |
| 30-49  | 9/61                  | 0.8 (0.3-1.7)   | 7/55                     | 0.6 (0.3-1.6)        | 2/6                       | 2.1 (0.3-16.6) <sup>‡</sup>   |
| $\geq 50$  | 13/65                 | 1.0 (0.5-2.2)   | 7/54                     | 0.6 (0.2-1.7)        | 6/11                      | 4.0 (0.8-20.8)                |

\*Includes *ALAD1-2* heterozygotes and *ALAD2-2* homozygotes.

<sup>†</sup>Models using exposure categories were adjusted for age and sex only.

<sup>‡</sup>Number of exposed cases or controls is less than five.

<sup>§</sup>Models of underlying continuous variables were adjusted for matching factors: age, sex, race, hospital, and distance of residence from hospital.

<sup>||</sup>Estimated airborne lead concentration for the job with highest exposure in the patient's work history.

**Table 4. Estimated risk of brain tumors with lead exposure after exclusion of proxy interviews, after 10-year exposure lag, and separate analysis for glioblastoma (NCI Brain Tumor Study, 1994-1998)**

|  | Cases/<br>controls (n) | Overall,<br>OR (95% CI) | Cases/<br>controls (n) | ALAD1 individuals,<br>OR (95% CI) | Cases/<br>controls (n) | ALAD2 individuals,<br>OR (95% CI)* |
|--|------------------------|-------------------------|------------------------|-----------------------------------|------------------------|------------------------------------|
| <b>Meningioma</b>  |                        |                         |                        |                                   |                        |                                    |
| Ever exposed to lead <sup>†</sup>                          |                        |                         |                        |                                   |                        |                                    |
| No <sup>‡</sup>  |                        | 1.0 (reference)         |                        | 1.0 (reference)                   |                        | 1.0 (reference)                    |
| Yes, excluding proxy interviews                            | 44/277                 | 0.8 (0.5-1.3)           | 20/159                 | 0.5 (0.3-1.1)                     | 12/27                  | 2.8 (0.7-10.3)                     |
| Yes, with 10-year lag                                      | 46/264                 | 0.9 (0.5-1.3)           | 21/151                 | 0.6 (0.3-1.1)                     | 13/23                  | 3.7 (0.9-14.8)                     |
| Cumulative exposure to lead,<br>excluding proxy interviews |                        |                         |                        |                                   |                        |                                    |
| $\beta$ , P  |                        | 0.003, 0.04             |                        | 0.002, 0.5                        |                        | 0.016, 0.01                        |
| OR for a 50 $\mu\text{g}/\text{m}^3\text{-y}$ increase     |                        | 1.16                    |                        | 1.16                              |                        | 2.19                               |
| Cumulative exposure to lead, with 10-year lag              |                        |                         |                        |                                   |                        |                                    |
| $\beta$ , P  |                        | 0.003, 0.05             |                        | 0.00, 1.0                         |                        | 0.008, 0.05                        |
| OR for a 50 $\mu\text{g}/\text{m}^3\text{-y}$ increase     |                        | 1.16                    |                        | 1.00                              |                        | 1.46                               |
| <b>Glioma</b>  |                        |                         |                        |                                   |                        |                                    |
| Ever exposed to lead <sup>†</sup>                          |                        |                         |                        |                                   |                        |                                    |
| No <sup>‡</sup>  |                        | 1.0 (reference)         |                        | 1.0 (reference)                   |                        | 1.0 (reference)                    |
| Yes, excluding proxy interviews                            | 156/277                | 0.8 (0.6-1.1)           | 98/159                 | 0.7 (0.5-1.0)                     | 20/27                  | 0.6 (0.2-1.8)                      |
| Yes, with 10-year lag                                      | 148/254                | 0.9 (0.7-1.2)           | 91/144                 | 0.8 (0.5-1.1)                     | 20/22                  | 1.1 (0.4-3.1)                      |
| Yes, glioblastoma only                                     | 106/287                | 0.9 (0.6-1.3)           | 62/166                 | 0.9 (0.6-1.6)                     | 12/28                  | 0.6 (0.2-2.6)                      |
| Cumulative exposure to lead,<br>excluding proxy interviews |                        |                         |                        |                                   |                        |                                    |
| $\beta$ , P  |                        | 0.0, 1.0                |                        | -0.001, 0.6                       |                        | 0.004, 0.3                         |
| OR for a 50 $\mu\text{g}/\text{m}^3\text{-y}$ increase     |                        | 1.00                    |                        | 0.97                              |                        | 1.23                               |
| Cumulative exposure to lead, with 10-year lag              |                        |                         |                        |                                   |                        |                                    |
| $\beta$ , P  |                        | 0.0, 0.9                |                        | -0.002, 0.3                       |                        | 0.002, 0.5                         |
| OR for a 50 $\mu\text{g}/\text{m}^3\text{-y}$ increase     |                        | 1.00                    |                        | 0.93                              |                        | 1.13                               |
| Cumulative exposure to lead, glioblastoma only             |                        |                         |                        |                                   |                        |                                    |
| $\beta$ , P  |                        | 0.0, 0.2                |                        | 0.001, 0.5                        |                        | 0.002, 0.4                         |
| OR for a 50 $\mu\text{g}/\text{m}^3\text{-y}$ increase     |                        | 1.1                     |                        | 1.05                              |                        | 1.11                               |

\*Includes ALAD1-2 heterozygotes and ALAD2-2 homozygotes.

<sup>†</sup>Models were adjusted for matching factors: age, sex, race, hospital, and distance of residence from hospital.<sup>‡</sup>Numbers of cases and controls not provided for reference category because the numbers change depending on the analysis (exclusion of proxy/10-year lag/glioblastoma only).

Although prior evidence would not have predicted a clear direction of risk, *a priori* biological and functional considerations indicated a possible association between lead exposure, ALAD G177C polymorphism, and brain tumor risk. The

relationship between lead and meningioma was modest overall, but notably elevated risks were observed for ALAD2 carriers with substantial lead exposure. Although these findings should be interpreted with caution given the small

**Table 5. Main effect of ALAD genotype on risk of meningioma by lead exposure status: OR and 95% CI of meningioma, NCI Brain Tumor Study, 1994-1998**

|   | ALAD1, cases/controls (n) | ALAD2, cases/controls (n)* | OR (95% CI)                   |
|---|---------------------------|----------------------------|-------------------------------|
| Ever exposed to lead <sup>†</sup>                                 |                           |                            |                               |
| No  | 95/254                    | 22/57                      | 1.1 (0.6-2.0)                 |
| Yes   | 21/166                    | 13/28                      | 4.2 (1.7-10.4)                |
| Highest exposure to lead ( $\mu\text{g}/\text{m}^3$ )             |                           |                            |                               |
| <10   | 95/254                    | 22/57                      | 1.0 (0.6-2.0)                 |
| 10-29   | 7/57                      | 5/11                       | 5.1 (0.6-43.2)                |
| 30-49   | 7/55                      | 2/6                        | 1.6 (0.1-21.2) <sup>‡</sup>   |
| $\geq 50$   | 7/54                      | 6/11                       | 5.5 (0.9-32.6)                |
| Cumulative exposure to lead ( $\mu\text{g}/\text{m}^3\text{-y}$ ) |                           |                            |                               |
| 0   | 88/238                    | 22/52                      | 1.1 (0.6-2.0)                 |
| 1-49  | 19/103                    | 4/19                       | 1.1 (0.2-4.8) <sup>‡</sup>    |
| 50-99   | 3/35                      | 4/10                       | 7.8 (0.7-91.2) <sup>‡</sup>   |
| $\geq 100$  | 6/44                      | 5/4                        | 32.4 (1.9-560.9) <sup>‡</sup> |
| Average exposure to lead ( $\mu\text{g}/\text{m}^3$ )             |                           |                            |                               |
| 0   | 88/238                    | 22/52                      | 1.1 (0.6-2.0)                 |
| 1-4   | 16/81                     | 3/21                       | 0.7 (0.1-4.8) <sup>‡</sup>    |
| 5-9   | 9/84                      | 6/9                        | 17.3 (2.5-120.1)              |
| $\geq 10$   | 3/17                      | 4/3                        | $\infty$                      |

NOTE: Models adjusted for age and sex only, unless otherwise specified. Each row represents a separate model.

\*Includes ALAD1-2 heterozygotes and ALAD2-2 homozygotes.

<sup>†</sup>Adjusted for all matching factors: age, sex, race, hospital, and distance of residence from hospital.<sup>‡</sup>Number of exposed cases or controls is less than five.

sample size for gene-environment evaluations, our results add to the evidence that tumorigenicity is yet another important adverse physiologic effect of lead, in addition to its known effects on the hematopoietic, gastrointestinal, urinary, cardiovascular, and nervous systems.

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