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Lead and cognitive function in *ALAD* genotypes in the third National Health and Nutrition Examination Survey

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ABSTRACT

The relationship between the blood lead concentration and cognitive function in children and adults with different *ALAD* genotypes who participated in the third National Health and Nutrition Examination Survey was investigated. The relationship between blood lead and serum homocysteine concentrations was also investigated. In children 12 to 16 years old, no difference in the relationship between cognitive function and blood lead concentration between genotypes was found. In adults 20 to 59 years old, mean reaction time decreased as the blood lead concentration increased in the *ALAD* rs1800435 CC/CG group. This represents an improvement in performance. In adults 60 years and older, no difference in the relationship between cognitive function and blood lead concentration between genotypes was found. The serum homocysteine concentration increased as the blood lead concentration increased in adults 20 to 59 years old and 60 years and older, but there were no differences between genotypes. The mean blood lead concentration of children with the *ALAD* rs1800435 CC/CG genotype was less than that of children with the GG genotype.

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1. Introduction

The purpose of this study was to determine if a single nucleotide polymorphism of *ALAD*, rs1800435, affects the relationship between the blood lead concentration and cognitive function in the children and adults participating in the third National Health and Nutrition Examination Survey (NHANES III). The relationship between blood lead and serum homocysteine concentrations was also investigated. The concentration of serum homocysteine increases as the concentration of blood lead increases in older adults [41], and there is evidence that homocysteine is neurotoxic [32].

ALAD is the gene for δ-aminolevulinic acid dehydratase (ALAD), which is a catalyst for the second step in heme synthesis in which two molecules of aminolevulinic acid are combined to form one molecule of porphobilinogen [43]. In humans, ALAD requires zinc in order to be active [18]. Lead inhibits the activity of ALAD in erythrocytes [24], resulting in increases in the concentrations of aminolevulinic acid in blood [49] and urine [42]. There is evidence that aminolevulinic acid is neurotoxic [45].

In humans, *ALAD* is located on chromosome 9. One of its single nucleotide polymorphisms, rs1800435, occurs at nucleotide 13 in exon 4, where guanine (G) is changed to cytosine (C). This results in a change in amino acids from lysine (K) to asparagine (N) at position 68 of ALAD. The two alleles, C and G, result in three genotypes, C homozygotes (CC), heterozygotes (CG), and G homozygotes (GG). The G allele is often referred to as *ALAD* 1 and the C allele as *ALAD* 2. Information about the gene and its single nucleotide polymorphisms can be found in the SNP500Cancer database (http://snp500cancer.nci.nih.gov/home_1.cfm).

In adults exposed to lead, those with the C allele have been found to have blood lead concentrations greater than G homozygotes. Factory workers with the CC/CG genotype had a mean blood lead concentration (47.0 μg/dl) that was greater than those with the GG genotype (38.4 μg/dl) [51]. Lead smelter workers with the CC/CG genotype had a mean blood lead concentration (25.18 μg/dl) that was greater than those with the GG genotype (22.85 μg/dl) [15]. In adults with lower concentrations, the opposite has been found. Men who participated in the Normative Aging Study with the CC/CG genotype had a mean blood lead concentration (5.7 μg/dl) that was less than men with the GG genotype (6.3 μg/dl) [25]. In children environmentally exposed to lead, those with the CC/CG genotype were found to have a mean blood lead concentration (27.1 μg/dl) that was greater than those with the GG genotype (19.5 μg/dl) [51].

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In a study of lead smelter workers [3], the percentage of lead in erythrocytes bound to ALAD was greater in the CC/CG group (84%) as compared to the GG group (81%). This indicates that ALAD may have a higher affinity for lead in those with the C allele, or that there may be more ALAD in their erythrocytes. The median blood lead concentration of the CC/CG group was 28.80 μg/dl and that of the GG group was 29.42 μg/dl. In the group of unexposed, control workers in this study, the percentage of lead in erythrocytes bound to ALAD was not different between the CG (79%) and GG (79%) groups. The median blood lead concentration of the CG group was 3.73 μg/dl and that of the GG group was 3.94 μg/dl.

Gibbs et al. [19] estimated the affinity of lead for ALAD using protein fluorescence ($K_d = 26.5$ μM) and steady state kinetics ($K_d = 32.6$ μM). No studies comparing the affinities of lead for ALAD between the *ALAD* genotypes were found.

Exposure to lead increases the amount of ALAD. In a group of ingot and battery factory workers [17], the mean concentration of ALAD in erythrocytes (92.32 μg/ml) was greater than the mean concentration in a group of control workers (54.1 μg/ml). The mean blood lead concentration of the exposed group was 41.2 μg/dl and that of the control group was 5.6 μg/dl. In accumulator factory workers [7], there was a positive correlation between the amount of ALAD in the blood and the blood lead concentration, with the blood lead concentration ranging from 30 to 75 μg/dl. In rat liver, there is evidence that lead exposure increases the concentration of ALAD by inducing transcription [16]. No studies comparing the concentrations of ALAD between the *ALAD* genotypes were found.

Sithisarankul et al. [46] found that men who worked in battery plants and who had the CG genotype had a lower median concentration of aminolevulinic acid in plasma (11.6 ng/ml) than men with the GG genotype (14.8 ng/ml). This indicates that lead may have less of an inhibitory effect on ALAD in those with the C allele. The median blood lead concentration of the CG group was 26.3 μg/dl and that of the GG group was 31.4 μg/dl.

In workers exposed to lead, statistically significant differences in ALAD activity have not been found between C homozygotes or heterozygotes and G homozygotes when activity is measured either as the amount of porphobilinogen per unit of time per concentration of red blood cells [11,54] or as the amount of aminolevulinic acid per unit of time per concentration of red blood cells [40,48].

2. Methods

2.1. Subjects

The subjects in NHANES III were civilian, non-institutionalized persons in the United States 2 months of age or older. They were selected using a complex, multistage sample design. The subjects included in this analysis were from the second phase of the survey conducted from 1991 to 1994. Three age groups were included based on the cognitive tests that were administered, children 12 to 16 years old ($n = 842$), adults 20 to 59 years old ($n = 2093$), and adults 60 years and older ($n = 1799$). Persons who were older than 90 years had their age coded as 90 to protect their identities.

2.2. Blood lead

Venous blood samples were taken at mobile examination centers or during home examinations given to persons who could not go to a mobile examination center. Blood lead was measured by atomic absorption spectrometry in persons one year and older. The limit of detection for the blood lead measurements was 1 μg/dl. Values below the limit of detection were assigned a value of 1 μg/dl divided by the square root of two. Details about the measurement of blood lead and the other blood measurements can be found in the NHANES III laboratory manual [21].

2.3. Serum homocysteine

Serum homocysteine was measured in persons 12 years and older during the second phase of the survey. It was measured by using reverse-phase high-performance liquid chromatography and fluorescence detection. The assay used measures both the reduced and oxidized forms of homocysteine. The limit of detection was 0 μmol/l.

2.4. Genotyping

Cell lysates were made from immortalized cell lines prepared from the white blood cells of consenting participants, 12 years and older, who were examined at a mobile examination center during the second phase of the survey. The lysates were supplied by the National Center for Health Statistics and the National Center for Environmental Health. The Core Genotyping Facility of the National Cancer Institute genotyped *ALAD* rs1800435 using lysates containing 5 ng of DNA in 5 μl TaqMan® 5′ nuclease reactions (Applied Biosystems, Foster City, California). Water controls and DNA samples with known genotypes, purchased from Coriell Cell Repositories (Camden, New Jersey), were included on each plate. Further details about the genotyping can be found in Chang et al. [10]. As reported in Chang et al. [10], no deviation from Hardy–Weinberg equilibrium was detected in each of three race-ethnicity groups (Non-Hispanic white, $p = 0.63$; Non-Hispanic black, $p = 0.48$; Mexican American, $p = 0.69$) for *ALAD* rs1800435.

2.5. WISC-R and WRAT-R cognitive tests

Two components of the Wechsler Intelligence Scale for Children-Revised (WISC-R, The Psychological Corporation, San Antonio, Texas), block design and digit span, and two components of the Wide Range Achievement Test-Revised (WRAT-R, Jastak Associates, Inc., Wilmington, Delaware), reading and arithmetic, were administered to children 6 to 16 years old. Age was determined at the time the components were administered at a mobile examination center. Only children 12 to 16 years old were included in the analysis, because the younger children were not genotyped. Age standardized scores were used.

2.5.1. Block design

Children put together red and white blocks in a pattern according to displayed models and displayed patterns on cards. There were 11 designs. There was a time limit for each design. Bonus points were awarded for designs 4 to 11 if they were completed before the time limit.

2.5.2. Digit span

Children were read sequences of 3 to 9 numbers and asked to repeat them as heard (digits forward) or in reverse order (digits backward). There were two trials for each sequence length, each using a different sequence. The sequence length was increased until both trials of a sequence length were not repeated correctly. Digits forward was administered first, followed by digits backward.

2.5.3. Reading

The reading subtest consisted of a pre-reading part and a formal reading part. In the pre-reading part, a child was asked to name 2 letters in a printed name, to identify 10 letters in a list of letters, and to name 13 printed letters. In the formal reading part, the child was asked to pronounce up to 75 or 74 words, until 10 consecutive errors were made. There are two levels of the test. Level 1 was given to children 5 to 11 years old, and level 2 was given to children 12 years and older.

2.5.4. Arithmetic

The arithmetic or 'math' subtest consisted of written and oral parts. The written part was administered first. A child was asked to