LEAD AND COGNITIVE FUNCTION IN *ALAD* GENOTYPES IN NHANES III

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Introduction

Lead is a neurotoxic metal whose pharmacokinetics vary in persons with different aminolevulinic acid dehydratase (*ALAD*) genotypes. The genotypes may modify the effect of lead on cognitive function by altering the amount of lead in nervous tissue or by altering biochemical pathways that produce other neurotoxic substances. The *ALAD* genotypes are summarized in Table 1.

Table 1. Single nucleotide polymorphisms (SNPs) of *ALAD*

Gene Symbol	Gene Name	Gene Position	dbSNP ID	Nucleotide change	Amino acid change	Gene product function	Effect on lead exposure
ALAD	Aminolevulinate, delta-, dehydratase	9q33.1	rs1800435	Ex4+13G>C	K68N	ALAD catalyzes the condensation of two molecules of δ-aminolevulinic acid in the second step in the porphyrin and heme biosynthetic pathway.	ALAD activity is inhibited by lead. The ALAD N68 variant binds lead more effectively than the ALAD K68 variant and modifies tissue distribution of lead in the body. Persons with ALAD N68 have higher blood lead levels, lower plasma δ-aminolevulinic acid levels, less efficient uptake of lead into bone and altered lead toxicities. [1]

The objective of this work was to determine if genetic variants in *ALAD* affect the relationships between blood lead levels and cognitive function in children and adults participating in the third National Health and Nutrition Examination Survey (NHANES III).

Methods

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 the analysis were from the second phase of the survey conducted from 1991 to 1994. Three age groups were used based on the cognitive tests that were administered, children 12 to 16 years old (n = 840), adults 20 to 59 years old (n = 2090), and adults 60 years and older (n = 1796).

Blood Lead

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.

Genotyping

Cell lysates were made from immortalized cell lines prepared from white blood cells from consenting participants 12 years and older, and 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 assay) reactions (Applied Biosystems, Foster City, California).

WISC-R and WRAT-R Cognitive Tests

Two components of the Wechsler Intelligence Scale for Children-Revised (WISC-R), digit span and block design, and two components of the Wide Range Achievement Test-Revised (WRAT-R), math and reading, 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 present analysis, because the younger children were not genotyped. Age standardized scores were used in the analysis.

Neurobehavioral Tests

Three neurobehavioral tests were administered to 20 to 59 year old adults at a mobile examination center. The three tests are components of the Neurobehavioral Evaluation System 2 (NES2, Neurobehavioral Systems, Inc., Atlanta, Georgia).

Simple reaction time. Subjects pressed a button whenever a solid square was displayed in the center of the computer screen. A total of 50 trials were administered to each subject. The mean reaction time (ms) of trials 11 through 50 was calculated.

Symbol-digit substitution. On the upper half of the computer screen subjects were presented with a grid that paired one of nine different symbols with one of the digits from 1 to 9. A similar grid was displayed on the bottom half of the screen with the same symbols presented in a scrambled order and the spaces for the corresponding digits left blank. Subjects entered the matching digit for each symbol. Four test trials were conducted with a different pairing of digits and symbols on each trial. The total latency was recorded for each trial and did not include the time it took to respond to the first item. The mean total latency (s) of four trials was calculated.

Serial digit learning. Subjects were presented with a series of digits displayed one at a time on the computer screen. After all the digits were displayed, subjects entered the sequence of numbers in the order in which they were presented using the numeric keys on the keyboard. The same eight-digit sequence was displayed on each trial. Testing continued until the subject responded correctly on two consecutive trials or until the subject attempted eight trials. The number of trials to reach the criterion was recorded.

Story Recall

Adults 60 years and older were administered a story recall test, either at a mobile examination center or during the home examination interview. A brief story was read by an interviewer to a subject, followed by a brief waiting period. Then the subject was asked to tell the story back to the interviewer. There were six ideas that were recorded as recounted or not recounted. The number of correctly recounted ideas was used as a measure of performance.

Statistical Analysis

The computer program SUDAAN® (Release 9.0.1, Research Triangle Institute, Research Triangle Park, North Carolina) was used to analyze the survey data.

Regression models were used to test for differences in mean blood lead levels between genotypes. No covariates were included. Regression analyses were performed between measures of cognitive function and the $\log_{10}$ blood lead levels adjusted for covariates. The genotype x $\log_{10}$ blood lead interaction was included in each model in order to test for the interaction and estimate the slopes for each genotype.

Covariates for the 12 to 16 year old children were sex, education of family reference person, family income, race-ethnicity, and test language. For the 20 to 59 year old adults, age, sex, education, family income, race-ethnicity, computer or video game familiarity, alcohol use in the last three hours, and test language were used. For the adults 60 years and older, age, sex, education, family income, race-ethnicity, and test language were used.

Results

Table 2. Blood lead concentration by age group and genotype

Age group (years)	Genotype	Blood lead (µg/dl) n	M	SE	LCL	UCL	Main effect F	p
12-16		840	1.95	0.16	1.63	2.27		
	ALAD rs1800435						11.55	0.0025
	CC/GC	**67**	**1.38**	**0.11**	**1.16**	**1.61**		
	GG	**751**	**2.03**	**0.18**	**1.66**	**2.40**		
20-59		2090	2.85	0.16	2.53	3.18		
	ALAD rs1800435						12.22	0.0019
	CC/GC	**161**	**2.34**	**0.26**	**1.79**	**2.89**		
	GG	**1835**	**2.92**	**0.16**	**2.60**	**3.25**		
60+		1796	4.02	0.08	3.85	4.19		
	ALAD rs1800435						0.09	0.7664
	CC/GC	203	3.96	0.26	3.43	4.49		
	GG	1548	4.05	0.10	3.85	4.25		

In children 12 to 16 years old and adults 20 to 59 years old (Table 2), the mean blood lead level of *ALAD* CC/GC group was less than the mean of the GG group.

Table 3. Slopes for test performance and $\log_{10}$ blood lead (µg/dl) in children 12 to 16 years old

Test	Genotype	Slope	SE	LCL	UCL	t	p	Interaction F	p
Math		**-11.08**	**4.55**	**-20.49**	**-1.67**	**-2.43**	**0.0231**		
	ALAD rs1800435							0.13	0.7169
	CC/GC	-16.37	17.88	-53.36	20.63	-0.92	0.3695		
	GG	-9.86	4.76	-19.71	-0.01	-2.07	0.0498		
Reading		**-12.77**	**3.18**	**-19.35**	**-6.20**	**-4.02**	**0.0005**		
	ALAD rs1800435							0.41	0.5275
	CC/GC	-19.29	11.95	-44.01	5.43	-1.61	0.1201		
	GG	-11.80	3.06	-18.13	-5.47	-3.86	0.0008		
Block design		**-1.50**	**0.58**	**-2.69**	**-0.30**	**-2.59**	**0.0165**		
	ALAD rs1800435							0.54	0.4707
	CC/GC	-2.65	1.64	-6.04	0.75	-1.61	0.1206		
	GG	-1.31	0.63	-2.61	-0.01	-2.08	0.0491		
Digit span		**-1.86**	**0.53**	**-2.96**	**-0.77**	**-3.53**	**0.0018**		
	ALAD rs1800435							0.44	0.5136
	CC/GC	-3.14	2.29	-7.88	1.60	-1.37	0.1835		
	GG	-1.67	0.49	-2.67	-0.67	-3.44	0.0022		

In children 12 to 16 years old (Table 3), performance on the cognitive tests declined as blood lead level increased.

Table 4. Slopes for neurobehavioral test performance and $\log_{10}$ blood lead (µg/dl) in adults 20 to 59 years old

Test Variable	Genotype	Slope	SE	LCL	UCL	t	p	Interaction F	p
Simple reaction time									
Mean reaction time (ms)		-6.33	7.10	-21.03	8.36	-0.89	0.3820		
	ALAD rs1800435							4.28	0.0499
	CC/GC	**-38.47**	**17.83**	**-75.36**	**-1.58**	**-2.16**	**0.0417**		
	GG	-2.34	7.58	-18.03	13.34	-0.31	0.7601		
Symbol-digit substitution									
Mean total latency (s)		0.93	0.55	-0.21	2.07	1.69	0.1054		
	ALAD rs1800435							1.89	0.1825
	CC/GC	2.72	1.46	-0.30	5.74	1.86	0.0752		
	GG	0.68	0.56	-0.47	1.83	1.23	0.2319		
Serial digit learning									
Trials to criterion		-0.02	0.29	-0.62	0.59	-0.06	0.9556		
	ALAD rs1800435							1.29	0.2679
	CC/GC	-0.77	0.72	-2.25	0.71	-1.08	0.2934		
	GG	0.06	0.28	-0.52	0.64	0.22	0.8316		

In adults 20 to 59 years old (Table 4), mean reaction time decreased as blood lead level increased in the *ALAD* CC/GC group. This represents an improvement in performance.

Table 5. Slopes for story recall test performance and $\log_{10}$ blood lead (µg/dl) in adults 60 years and older

Variable	Genotype	Slope	SE	LCL	UCL	t	p	Interaction F	p
Number correct		0.05	0.15	-0.26	0.37	0.34	0.7345		
	ALAD rs1800435							3.15	0.0890
	CC/GC	-0.88	0.55	-2.02	0.26	-1.60	0.1242		
	GG	0.16	0.17	-0.19	0.52	0.95	0.3531		

In adults 60 years and older (Table 5), performance on the story recall test was not related to blood lead level, overall or by genotype.

Discussion

In contrast to our results, factory workers (44 µg/dl) and children (26 µg/dl) with the *ALAD* CC/GC genotype that were exposed to lead had median blood lead levels 9 and 11 µg/dl higher than those with the GG genotype [2].

Blood lead may decrease reaction times in adults by altering the concentration of δ-aminolevulinic acid, which can bind to the receptors of γ-aminobutyric acid, an inhibitory neurotransmitter in the central nervous system [3].

References

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