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Lead and cognitive function in *VDR* 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 *VDR* 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, performance on the digit span and arithmetic tests as a function of the blood lead concentration varied by *VDR* rs2239185 and *VDR* rs731236 genotypes. Decreases in performance occurred in some genotypes, but not in others. In adults 20 to 59 years old, performance on the symbol-digit substitution test as a function of the blood lead concentration varied by *VDR* rs2239185–rs731236 haplotype. In the 12 to 16 year old children and adults 60 or more years old, the relationship between the serum homocysteine and blood lead concentrations varied by *VDR* genotype. The mean blood lead concentrations of the children and adults did not vary by *VDR* genotype.

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1. Introduction

The purpose of this study was to determine if single nucleotide polymorphisms of *VDR*, rs2239185 and rs731236, affect 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 [38], and there is evidence that homocysteine is neurotoxic [33].

VDR is the gene for the 1,25-dihydroxyvitamin D3 receptor (VDR), a nuclear hormone receptor. Gene expression is regulated by 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$) when it binds to VDR and transcription is either activated [36] or inhibited [25]. $1,25(OH)_2D_3$ plays a role in calcium homeostasis [4], but it also has effects in tissues that are not related to calcium homeostasis [44]. Walters et al. [45] hypothesized that $1,25(OH)_2D_3$ regulates the intracellular concentrations of calcium in these tissues. VDR has been found in many regions of the human brain [17]. There is also evidence for a vitamin D receptor in cell membranes that mediates effects of vitamin D that occur faster than transcription can take place [35].

In humans, *VDR* is located on chromosome 12. Two single nucleotide polymorphisms of *VDR* are considered in this paper. The first, rs2239185, occurs at nucleotide –3968 of intron 8, where thymine (T) is changed to cytosine (C). The two alleles, C and T, result in three genotypes, C homozygotes (CC), heterozygotes (CT), and T homozygotes (TT). The second, rs731236, is at nucleotide 32 of exon 11, where thymine is changed to cytosine. This results in no change in the amino acid isoleucine (I) at position 352 of VDR. The two alleles, C and T, result in three genotypes, C homozygotes (CC), heterozygotes (CT), and T homozygotes (TT). This polymorphism is also referred to as *Taq*I, with alleles T and t, corresponding to T and C above. Information about the gene and its single nucleotide polymorphisms can be found in the SNP500Cancer database (http://snp500cancer.nci.nih.gov/home_1.cfm).

Workers in a lead-acid battery manufactory with different *VDR* rs731236 genotypes were found not to have statistically significantly different blood lead concentrations [10]. The means were 35.8 (CC), 22.09 (TC), and 24.14 (TT) μg/dl.

Lead can interact with calcium and proteins in nerve cells. Lead inhibits the flow of calcium through voltage-dependent calcium channels [1]. Lead affects the amount of calcium in the mitochondria in synaptosomes [41]. Lead can bind to calmodulin and activate it [20]. Lead can inhibit adenylate cyclase activity [16]. Lead can activate

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protein kinase C [32]. Lead can inhibit Na^+–K^+ ATPase from synaptic plasma membranes [28].

$1,25(OH)_2D_3$ can affect the expression and activity of proteins that interact with lead. $1,25(OH)_2D_3$ inhibits the expression of voltage-dependent calcium channels in cultured hippocampal neurons [3]. $1,25(OH)_2D_3$ affects the calcium content and fluxes in mitochondria of skeletal muscle [39]. $1,25(OH)_2D_3$ can stimulate calmodulin synthesis in myoblasts [14]. $1,25(OH)_2D_3$ can inhibit adenylate cyclase activity in osteoblastic cells [9]. $1,25(OH)_2D_3$ increases the activity of protein kinase C in neuroblastoma cells [43]. $1,25(OH)_2D_3$ can induce the expression of the β1 subunit of Na^+–K^+ ATPase in myelomonocytic cells [2].

VDR polymorphisms could affect the relationship between the blood lead concentration and cognitive function by affecting the amount or activity of a protein that is regulated by vitamin D3 and that interacts with lead. Since *VDR* rs2239185 occurs in an intron and *VDR* rs731236 results in no change in amino acids, a difference between the genotypes of one of these polymorphisms may be due to a difference between the genotypes of a polymorphism on the same or another gene to which it is in linkage disequilibrium. A difference between genotypes could also be due to a difference in the expression of *VDR*. Polymorphisms in non-coding regions [26] and synonymous polymorphisms in coding regions [6] can affect the expression of a gene. The haplotypes of *VDR* rs2239185 and *VDR* rs731236 (CC, CT, TC, and TT) were also analyzed. A difference between the haplotypes of polymorphisms can represent the combined effect of the polymorphisms or it can represent the effect of a polymorphism to which the haplotype is in linkage disequilibrium [11].

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 [19].

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 *VDR* rs2239185 using lysates containing 5 ng of DNA in 5 μl TaqMan® 5′ nuclease reactions (Applied Biosystems, Foster City, California). The National Center for Environmental Health genotyped *VDR* rs731236 using 2 μl of eluate in 10 μl MBG Eclipse® 3′ hybridization triggered fluorescence reactions (Nanogen, Inc., Bothell, Washington) after purifying the DNA from the cell lysates using a ChargeSwitch® Direct 96 gDNA Kit (Invitrogen Corporation, Carlsbad, 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. [8]. Chang et al. [8] reported the results of the tests for deviation from Hardy–Weinberg equilibrium for *VDR* rs2239185 (non-Hispanic White, $p=0.00$; non-Hispanic Black, $p=0.98$; Mexican American, $p=0.85$) and *VDR* rs731236 (non-Hispanic White, $p=0.12$; non-Hispanic Black, $p=0.26$; Mexican American, $p=0.22$).

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 write down the answers to addition, subtraction, multiplication, and division problems. The oral part was administered after the written part. The child counted ducks, boxes, and dots, read numbers, showed fingers, said which number is more, and answered brief word problems that required addition and subtraction. 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.