NeuroToxicology 33 (2012) 37–43

Contents lists available at SciVerse ScienceDirect

NeuroToxicology

Modification by the genes ALAD and VDR of lead-induced cognitive effects in children

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ARTICLE INFO

Article history:
Received 11 July 2011
Accepted 30 October 2011
Available online 9 November 2011

Keywords:
Central nervous system
Neurotoxicity
Polymorphism
Lead
IQ
Gene–environment interaction

ABSTRACT

Lead has negative effect on cognitive functions in children. However, individuals differ in susceptibility. One possible explanation is a genetic predisposition. Polymorphisms in the δ-aminolevulinic acid dehydratase (*ALAD*) and the vitamin D receptor (*VDR*) genes may modify lead metabolism and neurotoxicity, but information regarding the central nervous system is very limited. The aim of the study was to determine whether *ALAD* and *VDR* polymorphisms modify blood lead (B-Pb), and the association between B-Pb and cognitive function (IQ) in children. In 2007–2010 a cohort of 175 children (age 6–10 years, mean 7.8) was recruited in Southern Poland, tested for IQ (Wechsler intelligence scale) and analyzed for B-Pb (range 9.0–221; mean 46.6 μg/L), *ALAD* (*Rsa*I, *Msp*I) and *VDR* (*Fok*I, *Bsm*I, *Taq*I) polymorphisms. *ALAD* or *VDR* genotypes were not associated with B-Pb. B-Pb was non-significantly negatively associated with full scale IQ ($r_S = -0.11$; $P = 0.14$), and significantly with performance subscale results ($r_S = -0.19$; $P = 0.01$). The *ALAD Rsa*I polymorphism modified the relationship between full scale IQ and B-Pb: *Rsa*I T carriers had a steeper slope compared to CC homozygote carriers (β coefficient −0.06 vs 0.32, respectively, *P* for interaction <0.001, adjusted for the child's age, mother's education and family income). This means that with increasing B-Pb with 1 μg/L, T carriers demonstrate 0.06 score lower IQ. For the *VDR Bsm*I, B carriers had a steeper slope than the bb homozygotes carriers (β coefficient −0.08 vs 0.16, respectively, *P* for interaction = 0.001), and similar effect was found for *Taq*I t carriers vs TT homozygotes (*P* for interaction = 0.02). For *ALAD Msp*I and *VDR Fok*I there was no significant modification. The *ALAD Rsa*I, *VDR Bsm*I and *Taq*I polymorphisms modified the relationship between IQ and B-Pb. Hence, there is a fraction of the population, which is particularly sensitive to lead neurotoxicity.

1. Introduction

Exposure to lead is a major risk for the developing central nervous system (CNS) in children (Bellinger, 2006; Kmiecik-Małecka et al., 2009; Lanphear et al., 2005). Lead exposure in childhood may have long-term influence on functioning in adulthood (Mazumdar et al., 2011). There is a large variation in the susceptibility to the effects of lead exposure. Some of these differences may be explained by differences in the genetic background (review by Nordberg et al., 2007). In particular, polymorphisms in the δ-aminolevulinic acid dehydratase gene *ALAD*, the product of which is the major lead-binding protein in blood, has been shown to affect blood lead level (B-Pb; Scinicariello et al., 2007; Zhao et al., 2007); *ALAD2* (the variant allele of polymorphism *Msp*1, also called rs1800435) carriers demonstrate higher blood lead concentration at higher exposures. *ALAD* modifies the toxic effects on hem synthesis and kidney function (Scinicariello et al., 2007; Tian et al., unpublished). Also, there might be genetic modification of lead toxicity on CNS – a protective effect in *ALAD*2 carriers on cognitive functions in children has been reported (Bellinger et al., 1994) and adult lead workers (Gao et al., 2010). Also, Krieg et al. (2009) observed a better reaction time in adult *ALAD*2 carriers, but no effect in children. Further, Rajan et al. (2007) recorded less effect on mood in *ALAD*2 carriers. On the other hand, there are reports on worse cognitive performance in *ALAD*2 carriers among elderly men (Rajan et al., 2008; Weuve et al., 2006).

Abbreviations: ALAD, δ-aminolevulinic acid dehydratase; B-Pb, blood lead concentration; CNS, central nervous system; IQ, cognitive function; VDR, vitamin D receptor; WISC-R, Wechsler intelligence scale for children – revised.

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doi:10.1016/j.neuro.2011.10.012

All these studies are based on modifications by polymorphism in *ALAD Msp*I (also called rs1800435); only one study assessed other polymorphic *ALAD* sites (Chia et al., 2007). For *ALAD Rsa*I they found some protection in C-variants on motor dexterity function in Singaporean lead workers. Thus, there is an obvious need for further studies of genetic susceptibility on CNS toxicity.

Though even less studied than *ALAD*, there are some indications that polymorphisms in the vitamin D receptor gene *VDR* also affect lead toxicokinetics (Nordberg et al., 2007). This may be because VDR is involved in calcium metabolism that interacts with lead: for *VDR Bsm*I (rs1544410), the bb genotype was associated with lower B-Pb (Rezende et al., 2008; Schwartz et al., 2000); for *VDR Fok*I (rs2228570) the f genotype was also associated with low B-Pb (Haynes et al., 2003; Rezende et al., 2008). However, the possibility that *VDR* genotypes may modify the toxicity of lead has been studied only occasionally, though one study on CNS toxicity suggests that the *VDR* polymorphism *Taq*I modifies the toxicity (Krieg et al., 2010).

There are only few studies reporting the genetic modification of lead neurotoxicity by *ALAD* and *VDR* and most of them refer to adults. We here report the study on genetic modification by *ALAD* and *VDR* polymorphisms on B-Pb and its relation to cognitive effects in children. Our hypothesis is that *ALAD2* (*Msp*I) carriers, due to stronger sequestering of blood lead and/or less effects of lead on the energy metabolism of nerve cells compared to *ALAD1* carriers, have less effects of lead on IQ. We also hypothesize that *ALAD Rsa*I and *VDR* genotype determine the neurotoxicity of lead due to differences in the bioavailability of lead.

2. Materials and methods

2.1. Subjects

In 2007–2010, a cohort of children from southern Poland was invited to participate (Table 1). Children were recruited among students of primary school (6–10 years old) located in the vicinity of industrial emitters of lead in Upper Silesia. The head of each school was contacted and detailed information on the study was passed to parents or caregivers. At the two-day examination days, the parent first completed a questionnaire (including information about sex of the child, age, weight, height, family income, mother's and father's education, as well as the child's medical history, development and habits), and a neurophysiological testing procedure of the child was then performed of which cognitive function test (IQ) with Wechsler intelligence test was one part. Also, blood was collected for genotype determination.

The final database comprised 300 subjects from 365 children invited to the study (the participation rate was 82%). Out of them, 186 were tested for IQ. The reasons for lower participation rate for the IQ test were several: some children did not come to the second test day including the IQ test; sometimes the psychologists were prohibited to perform the test due to other duties. We compared the children who performed with the children who did not perform the test for variables used in analyses (i.e. sex, age, blood lead level, socioeconomic factors, etc.) and there were no significant differences between those two groups, or with the whole cohort. In case of examination of siblings, only (randomly selected) one child from the family was included into final gene–environment interaction analyses, resulting in total number of analyzed non-related cases $N = 175$ included in further analysis. The population under study was homogenous with respect to ethnicity.

The study was approved by Bioethics Committee at the Institute of Occupational Medicine and Environmental Health, Sosnowiec, Poland. Written informed consent was obtained from one parent and oral consent from the child.

Table 1
Characteristics of the cohort.

Variables[a]	Values (total $N = 175$)
Boys (%)/girls	101 (57.7)/74 (42.3)
Age, mean (range)	7.8 (6–10)
Mother's education (%)	
Primary	19 (11)
Apprenticeship	37 (21)
Secondary	81 (47)
Bachelor degree	11 (6)
Master degree	26 (15)
Father's education (%)	
Primary	9 (5)
Apprenticeship	71 (43)
Secondary	66 (40)
Bachelor degree	7 (4)
Master degree	14 (8)
Income (%)	
Low	41 (26)
Moderate	75 (47)
High	44 (27)
Apgar score at birth, mean (range)	9.5 (5–10)
Birth weight (g), mean (range)	3296 (1500–4450)

[a] Missing data: Age = 1 (0.6%); Mother's education = 1 (0.6%), Father's education = 8 (4.6%), Incomes = 15 (8.6%), Apgar score = 9 (5,1%), Birth weight = 8 (4.6%).

2.2. Psychological testing

The children were tested for IQ with Wechsler intelligence scale for children – revised (WISC-R, Wechsler, 1974) suitable for examining children aged 6–16 years. The multivariate statistical analysis of results was additionally adjusted for children's age. A version in Polish adapted by the Polish Society of Psychology (Matczak et al., 1997) was administered to the children. The test consists of 12 subtests divided into two scales – verbal scale, which is based upon child native language skills; and performance scale, based upon visual, spatial perception and motor abilities. Verbal IQ results from a number of test that represent information, similarities, arithmetic, vocabulary and comprehension tests; performance IQ consists of picture completion, picture arrangement, block design, object assembly and coding test. The full scale IQ result consists of verbal and performance IQ subset of tests.

Polish norms were standardized (Matczak et al., 1997). In our project WISC-R was performed and assessed by two qualified psychologists. The full-scale IQ (also referred to as global IQ), and performance IQ value were used for multivariate analyses.

2.3. Blood sampling

Two 4 ml samples of blood were obtained from the cubital vein into vacuum tubes (Vacuette®; Greiner-Bio, Frickhausen, Germany) containing either lithium heparin for lead determination, or K_3EDTA for genotype assessment. Blood samples were stored in $-20\,^{\circ}C$ until analyses.

2.4. Genetic analyses

DNA was extracted from peripheral whole blood using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden). The following SNPs were analyzed for *ALAD*: rs1139488 (also referred to as *Rsa*I), rs1800435 (also referred to as *Msp*I with *ALAD*1 and 2 as alleles); and for *VDR*: rs2228570 (also called *Fok*I), rs731236 (*Taq*I), and rs1544410 (*Bsm*I).

For *ALAD* rs1139488 and *VDR* rs1544410, assays based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) were used to determine the genotypes. The PCR was performed in a 12.5 μl reaction volume containing 0.2 μM of each primer (5′-AGGAGCCTTCCACAGCCGAAT-3′ and 5′-CCTTCCTTTTTCTGTTTGTATTGGAGAC-3′ for rs1139488; and