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Original Article

Relationship among maternal blood lead, ALAD gene polymorphism and neonatal neurobehavioral development

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Abstract: Lead is a widely used heavy metal that can affect children's nervous system development. ALAD gene polymorphism is associated with lead neurotoxicity. This study aimed to clarify the relationship among maternal blood lead, ALAD gene polymorphism, and neonatal neurobehavioral development through detecting maternal blood lead and ALAD gene polymorphism. 198 maternal and neonatal were selected as the research object. Graphite furnace atomic absorption method was applied to detect the maternal blood lead concentration. PCR-RFLP was used to detect ALAD genotype distribution. Neonatal NANB score was treated as effect indicator. SPSS was used for statistical analysis. The ALAD genotype was 181 cases (91.4%) for $ALAD_{11}$ and 17 cases (8.6%) for $ALAD_{12}$. ALAD allele frequency distribution accords with genetics Hardy-Weinberg balance ($P > 0.05$). Blood lead level in maternal with $ALAD_{12}$ genotype was significantly higher than with $ALAD_{11}$ genotype ($P < 0.01$). NANB score in high blood lead neonatal group was obviously lower than the low blood lead group ($P < 0.05$). Newborn's NANB score from the maternal with $ALAD_{11}$ genotype was lower than from the maternal with $ALAD_{12}$ genotype ($P < 0.01$). After ruling out the confounding factors influence by multiple linear regressions, ALAD gene polymorphisms had no significant correlation with neonatal NANB score ($P > 0.05$). ALAD gene polymorphism is associated with the blood lead level. Low level lead exposure in utero may cause newborn early neurobehavioral maldevelopment. Maternal ALAD gene polymorphism can affect early neonatal neurobehavioral development by influencing the blood lead level.

Keywords: ALAD, gene polymorphism, blood lead, newborn, neurobehavioral development

Introduction

Lead is a heavy metal that ubiquitous in the environment because of its non-biodegradability. It may harm to human health by entering the body through various pathways. A large number of studies have shown that lead can affect the body's blood system, nervous system, and digestive system. Children and professional people are at risk of lead exposure. Lead exposure on children's damage mainly manifests as affecting the nervous system development which result in affecting the children's intelligence [1-4]. Lead toxicity to the neural development is lack of threshold. As reported by Murata et al [5], blood lead concentrations less than 5 mg/dl may inhibit hemoglobin synthesis in adult and cause damage to children's intelligence. Therefore, identify the risk factor of lead toxicity to neural development plays an important role. The cause of lead poisoning is very clear, but the toxic effects in different individual performance are various. In the same exposure condition, some individuals appear lead poisoning, while some only show slightly higher blood lead. It implies that in the process of lead poisoning, genetic factors also play an important role in addition to environmental etiology. Researches show that ALAD gene polymorphism and lead poison have a relative relationship with blood lead level [6-8]. Therefore, ALAD gene polymorphism has relationship with lead liver and kidney toxicity and the damage to the hematopoietic system [9]. Recent study suggested that ALAD gene polymorphism has relationship with lead neurotoxicity. Sobin et al [10] reported that with the increase of blood lead level, ALAD2 associated with the enhancement of visual attention and working memory; Zheng et al [11] found ALAD gene polymorphism regu-

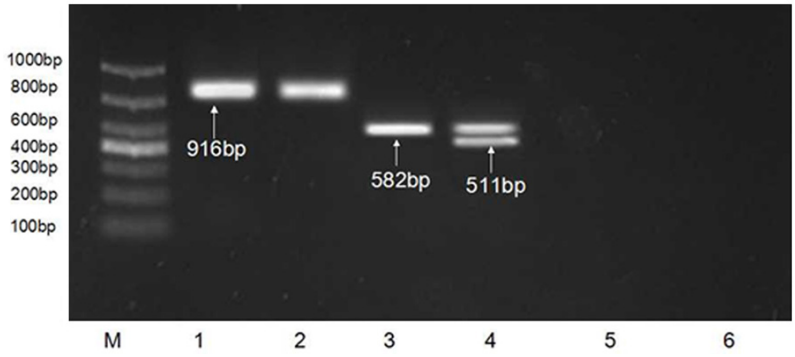

Figure 1. ALAD gene polymorphism PCR products and enzyme-digested products gel-imaging. M, DNA marker; 1 and 2, undigested PCR products; 3, $ALAD_{11}$ genotype; 4, $ALAD_{12}$ genotype; 5 and 6, negative control.

lates the relationship between lead and peripheral nerve toxicity, and it also correlates with the influence of lead on children's cognitive ability [12]. Therefore, this study selected maternal and neonatal as the research object from the same area. In order to investigate the relationship among the maternal blood lead, ALAD gene polymorphism, and neonatal neurobehavioral development, we detected maternal blood lead level and ALAD gene polymorphism, and rated on neonatal neurobehavioral development at the same time. It may provide clue for the susceptibility biomarkers involved in the influence of neurological development caused by lead toxicity, and provide the basis for studying the influence factors about neonatal neurobehavioral development.

Subjects and methods

Subjects

Physical healthy maternal and neonatal were selected from a Guangzhou maternal and child care service centre between January 1, 2013 and December 31, 2013 as the research objects. Questionnaire survey was used to collect the basic information of the maternal, and the venous blood was collected during the childbirth. Exclusion criteria: (1) maternal with long time lead exposure history, such as lead drugs and food, or leaded container and cosmetics, etc.; (2) maternal with smoking or drinking history; (3) the newborn with birth defects or premature; (4) maternal with drugs during pregnancy that may influence neonatal nervous system development, such as aminoglycoside drugs, antiepileptic drugs, and so on. 198 healthy maternal and mature newborns were collected. All of the maternal are the Han nationality, aged 25±0.53 years old.

The study protocol was approved by the Research Ethics Committee of our hospital, and all patients gave their informed consent before study commencement.

Blood sample collection

6.0 ml venous blood was collected during childbirth and maintained with heparin in two tubes. One was for DNA extraction, and another was for blood lead measurement.

Blood lead concentration measurement

Atomic absorption spectrometry was applied to measure the whole blood lead level using the Z-5000 atomic absorption spectrophotometer instrument according to the laboratory operating procedures. All of the equipment received lead-free treatment.

ALAD genotyping

Total genomic DNA was extracted from the peripheral blood. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) was applied for ALAD genotyping. PCR amplification primer was designed according to the e Hopkins [13] report. The cycling conditions consisted of an initial, single cycle of 10 min at 95°C; followed by 30 cycles of 60 s at 95°C, 35 s at 56°C, and 1 min at 72°C; and followed by 7 min at 72°C for extension. PCR amplification of ALAD gene fragment was digested by enzyme MspI, and the digested product was performed for agarose gel electrophoresis to judge the variation of ALAD genotype.

Pathogen of neonatal neurobehavioral inspection

Domestic accepted NBNA method was applied. The inspectors were trained by specialist and received assessment. The inspection started from the 3rd day after delivery.

Table 1. Neonatal neurobehavioral development score comparison in different blood lead level

Group	Cases	score					
		NBNA	Active ability	Passive muscular tension	Active muscular tension	Primitive reaction	General reaction
High lead group	99	38.25±1.71	11.41±0.84	7.80±0.43	7.23±0.64	5.90±0.31	5.91±0.27
Low lead group	99	38.85±1.53	11.60±0.81	7.88±0.42	7.45±0.56	5.95±0.32	5.97±0.26
t value		2.06	1.62	1.32	2.57	1.12	1.59
P value		0.01*	0.11	0.19	0.01*	0.27	0.11

*$P < 0.05$, **$P < 0.01$, compared with high lead group.

Table 2. Neonatal neurobehavioral development score comparison in different genotype

Group	Cases	score					
		NBNA	Active ability	Passive muscular tension	Active muscular tension	Primitive reaction	General reaction
$ALAD_{11}$	181	38.71±1.55	11.62±0.79	7.84±0.40	7.38±0.51	5.93±0.30	5.94±0.26
$ALAD_{12}$	17	36.84±1.87	10.30±1.13	7.82±0.45	6.90±0.72	5.89±0.33	5.93±0.28
t value		4.67	6.32	0.20	3.57	0.52	0.15
P value		0.000**	0.000**	0.84	0.000**	0.60	0.88

*$P < 0.05$, **$P < 0.01$, compared with $ALAD_{11}$ group.

Statistical analysis

All statistical analyses were performed using SPSS11.0 software (Chicago, IL). Goodness-of-fit chi-square test was used to detect Hardy Weinberg equilibrium. Rank sum test was applied for mean comparison of measurement data that do not obey the normal distribution; t test was applied for that obeys the normal distribution. Multiple linear regressions were used to eliminate the possible confounding factors. Inspection level of $\alpha = 0.05$.

Results

Maternal bloods lead level

The blood lead level of 198 maternal was 0.35±0.19 μmol/l, and the range was 0.10-1.37 μmol/l. The 25th, 50th, and 75th percentile was 0.23 μmol/l, 0.31 μmol/l, and 0.43 μmol/l, respectively.

ALAD gene polymorphism

ALAD PCR products and enzyme-digested products were shown in **Figure 1**. The ALAD genotype was 181 cases (91.4%) for $ALAD_{11}$ and 17 cases (8.6%) for $ALAD_{12}$. $ALAD_{22}$ was not found. The frequency distribution of $ALAD_1$ and $ALAD_2$ allele were 95.7% and 4.3%, respectively. ALAD allele frequency distribution accords with genetics Hardy-Weinberg balance ($\chi^2 = 0.40$, $P > 0.05$), indicating that the selected subjects has representativeness.

Blood lead level comparison in different genotype

The blood lead level in maternal with $ALAD_{11}$ genotype was 0.32±0.14 μmol/l, while it was 0.70±0.32 μmol/l in the maternal with $ALAD_{12}$ genotype. Blood lead level in maternal with $ALAD_{12}$ genotype was significantly higher than with $ALAD_{11}$ genotype ($P < 0.01$).

Neonatal neurobehavioral development score comparison in different blood lead level

The subjects were divided at the blood lead level of 0.31 μmol/l, which is the 50th percentile, into high lead and low lead group. NANB score in high blood lead neonatal group was obviously lower than the low blood lead group ($t = 2.06$, $P < 0.05$). High lead group exhibited significantly lower active muscle tension score than low lead group ($t = 2.57$, $P < 0.05$) (**Table 1**).

Neonatal neurobehavioral development score comparison in different genotype

Newborn's NANB score from the maternal with $ALAD_{11}$ genotype was higher than from the maternal with $ALAD_{12}$ genotype ($t = 4.67$, $P < 0.01$). Newborn in $ALAD_{11}$ genotype presented

higher neonatal behavior ability and active muscle tension score than in $ALAD_{12}$ genotype ($P < 0.01$). Multiple linear regression was performed based on exclude confounding factors such as maternal age, blood lead level, and ALAD genotype. It was found that ALAD gene polymorphisms had no significant correlation with neonatal NANB score ($P > 0.05$), while NANB score was obviously correlated with blood lead level ($P < 0.05$) (**Table 2**).

Discussion

δ-amino levulinic acid dehydratase (ALAD) is an important enzyme in the process of hemoglobin synthesis. ALAD gene polymorphism was first discovered by Battistuzzi [14]. It has two alleles named $ALAD_1$ and $ALAD_2$, and the two alleles both have dominant inheritance. $ALAD_2$ production is caused by the 177th base of the ALAD coding sequence appeared G-C transversion, which makes the electroneutral aspartyl change to electropositive lysine. It causes the negative charge in $ALAD_1$ higher than in $ALAD_2$, while the binding force to lead of $ALAD_{12}$ and $ALAD_{22}$ higher than $ALAD_{11}$, influencing the blood lead level [11]. Lead has neurotoxicity, and numerous studies have shown that low level of lead exposure may affect neonatal neurobehavioral development. Liu et al [15] reported that even the lead concentration as low as 5 μg/dl; it still can affect the neonatal neurobehavioral development. In addition, it has been reported that ALAD gene polymorphism can regulate lead neurotoxicity [10]. Thus, our study aimed to clarify the relationship among maternal blood lead, ALAD gene polymorphism, and neonatal neurobehavioral development through detecting maternal blood lead and ALAD gene polymorphism. Results suggested that, in the same exposure area, maternal carrying $ALAD_{12}$ genotype has higher blood lead level than that carrying $ALAD_{11}$ genotype. It indicated that ALAD gene polymorphism is associated with the blood lead level, which was consistent with the previous results. In 1986, Ziemsen et al. [16] first reported the relationship between ALAD polymorphism and lead toxicity. Workers carrying $ALAD_2$ allele had higher blood lead level, and the same results were also confirmed in the subsequent studies [17].

To clarify the relationship between maternal blood lead level and neonatal neurological development, we chose 0.31 μmol/l as the cut-off point of maternal blood lead level. Neonatal neurobehavioral development score in high lead group was lower than in low lead group, indicating that low level lead exposure in utero can affect the neonatal neurobehavioral development. As early as in 1997, Zhang had found that neonatal neurobehavioral development score was lower in the high lead group than the low lead group when choosing 0.48 μmol/l lead level in umbilical cord blood as the cut-off point [18]. Since then, Laughlin also reported that [19] lead exposure can affect the neonatal neurobehavioral development when using macaques as the research object. In recent studies, Liu, et al. [15] suggested that blood lead level during the first three months of pregnant was negatively correlated with neonatal NANB score. Our result is consistent with the abovementioned reports, further confirming that neonatal neurobehavioral development is influenced by environmental lead exposure.

Comparing the neonatal NANB score in different ALAD genotypes, it was found that newborn's NANB score from the maternal with $ALAD_{11}$ genotype was higher than from the maternal with $ALAD_{12}$ genotype. However, after ruling out the confounding factors influence, ALAD gene polymorphisms had no significant correlation with neonatal NANB score. Combined with the fact that ALAD gene polymorphism can affect the blood lead level, it showed that maternal ALAD gene polymorphism may is affect the neonatal NANB score by influencing the blood lead level. There is no research about the relationship between maternal ALAD gene polymorphism and neonatal neurobehavioral development. However, studies have shown that ALAD gene polymorphism is associated with lead neurotoxicity. Chia et al. [20] revealed that $ALAD_2$ allele may be a protective factor to lead neurotoxicity, and this result was confirmed by the later study [10, 21, 22]. The specific mechanism of its nerve protection is not yet clear and need further study.

Disclosure of conflict of interest

None.

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