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Association between delta-aminolevulinic acid dehydratase polymorphism and placental lead levels

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ABSTRACT

Lead inhibits the delta-aminolevulinic acid dehydratase (ALAD) activity and results in neurotoxic aminolevulinic acid accumulation in the blood. During pregnancy, lead in the maternal blood can easily cross the placenta. The aim of this study was to determine whether the maternal ALAD G177C polymorphism (rs1800435) was related to the placental lead levels. The study population comprised 97 blood samples taken from mothers to investigate ALAD G177C polymorphism and their placentas to measure lead levels. ALAD G177C polymorphism was detected by standard polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) technique and atomic absorption spectrometry (AAS) equipped with a graphite furnace and Zeeman background correction system was used for lead determination. The median placental lead levels for ALAD1-1, ALAD1-2 and ALAD2-2 genotypes were 7.54 μg/kg, 11.78 μg/kg and 18.53 μg/kg, respectively. Statistically significant association was found between the maternal ALAD G177C polymorphism and placental lead levels ($p < 0.05$). This study suggested that maternal ALAD G177C polymorphism was associated with placental lead levels.

1. Introduction

Lead, naturally occurring heavy metal, is a serious health problem (Yang et al., 2012). It enters human body through food, contaminated air and dust, and causes significant adverse effects on various tissues. Hematopoietic system is one of the targets of lead poisoning since most of the lead accumulates in erythrocytes (Onalaja and Claudio, 2000). The enzymes in biosynthetic pathway of heme are sensitive to the action of lead. Delta-aminolevulinic acid dehydratase (ALAD) activity, one of the enzymes of heme biosyntesis pathway, is accepted as an indicator of lead poisoning. Lead can inhibit ALAD activity stoichiometrically through the displacement of zinc that is essential for ALAD activity (Yang et al., 2012). Since Pb^{2+} is bigger than Zn^{2+}, it is not likely to interact with amino acid residues and substrates in the active center properly. The inhibition of ALAD by lead not only interrupts heme biosynthesis pathway but also causes to the accumulation of neurotoxic ALA, so it contributes to lead toxicity with two ways. In this case, the relation between ALAD and ALA is different from those which classical enzymes and substrates have (Rocha et al., 2012).

ALAD, also known as porphobilinogen syntase (PBGS) (Shaik and Jamil, 2008), is essential for aerobic organisms and it catalyses the condensation reaction of transubstantiating two ALA molecules into a PBG ring (Tanaka et al., 2011). Since it could condense 2 identical ALA substrates asymmetrically recognizing via its A and P zones known as binding pocket, ALAD is suggested as an unusual enzyme (Frere et al., 2002). The human ALAD possesses 8 monomers that each is ~37 kDa molecular weight; thus, total weight might vary between 252 to 280 kDa (Sakai, 2000). These 8 monomers generate 4 dimers which are active areas. Of 8 Zn^{2+} ions which are found in each monomers, 4 play roles in the catalysis and this monomer site that binds Zn^{2+} ions are termed Zn_A. The Zn_B site, where the remaining Zn^{2+} ions bind, participates in the catalysis as Lewis acid. It is thought that Zn_B takes charge in the stability of homooctameric protein configuration (Plewinska et al., 1991).

ALADs are limitless and cytoplasmic metalloenzymes (Fachinetto et al., 2006). They require either Mg^{2+}, Zn^{2+}, or both of them for catalysis (Frankenberg et al., 1999). Mammalian ALADs are in need of Zn^{2+} and thiol groups for maximal catalytic activity. Zn^{2+} ions participate in the stabilization of thiol groups. The vicinal cysteinyl residuals known as thiol groups on their active areas provide a coordination of essential Zn^{2+} ions and due to this vicinity ALADs become sensitive to oxidation (Rocha et al., 2012).

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ALAD gene is localized on chromosome 9q34. It is 15.9 kb length and possesses 11 coding and 2 noncoding locked exons named as 1_A and 1_B, respectively (Zheng et al., 2011). Promoter 1_A is functional in whole body, whereas promoter 1_B is only functional in the erythrocyte cells (Mijares et al., 2006). ALAD gene is polymorphic (Montenegro et al., 2006) and 8 gene variants were described on it (Skerfving and Bergdahl, 2007). ALAD rs1800435 polymorphism results from a G177C substitution due to a substitution of asparagines for lysine at amino acid position 59 in the coding region of the ALAD gene, thus leading to two alleles (ALAD1 and ALAD2) and three phenotypes (ALAD1-1, ALAD1-2, and ALAD2-2) (Shaik and Jamil, 2008; Zheng et al., 2011). Lysine is a positively charged amino acid, whereas asparagine is neutral; thus, ALAD2 produce more electronegative ALAD than ALAD1. This fact causes that lead ions with positive charge would bind more easily with the protein encoded by the ALAD2 allele rather than a protein encoded by the ALAD1 allele (Kelada et al., 2001). Furthermore, ALAD is a metalloprotein; thus, the substitution of charged lysine to neutral asparagine might lead to the alterations in enzyme's binding ability of not only lead but also some other metals such as zinc and magnesium (Van Bemmel et al., 2011).

Placenta is an immensely specialized organ originates during pregnancy. It supports normal growth and fetal development with fetal membrane and amniotic fluid (Kayaalti et al., 2011). Placenta has several functions such as nutrient absorption, waste excretion, gas transfer and hormonogenesis alone that an adult's various organs all together have (Van Der Aa et al., 1998). Xenobiotics such as drugs, pesticides, toxic metals and occupational chemicals can pass into placenta due to the circulation accompanied by nutrients and endogenous substrates (Pasanen, 1999). Albeit it serves as a barrier, placenta could not protect fetus against toxicants such as lead and cadmium (Kayaalti et al., 2011).

The aim of this study was to analyze whether maternal ALAD G177C gene polymorphism affects the placental lead levels. For this, we examined the frequency distribution of ALAD G177C (rs1800435; Gene ID: 210; Accession Number: NM_000031.5) genotypes and the association between maternal ALAD genotype and lead levels of placenta.

2. Materials and methods

2.1. Study subjects

The study population was included 97 mother and their placentas. The women in this study consisted of consecutive cases coming to the Gynecology Department of Ankara University's Faculty of Medicine. Mothers with gestational ages ≥36 weeks were screened in the hospital and asked to fill out a questionnaire, which included medical and dietary history, as well as data on occupational and potential environmental sources of metal exposure, and socioeconomic status. Healthy, non-smoking, non-anemic, non-diabetic mothers living in Ankara for more than 3 years without a history of alcohol, smoking, drug use or chronic disease were included in the study. A small questionnaire used to gather demographic information was also given to women. Only Turkish subjects were included in the study. Exclusion criteria was a medical history of renal failure, diabetes, carcinoma, diagnosed hepatic or cardiovascular diseases that may be related to possible heavy metal accumulation from environmental or occupational exposures. Informed consent was obtained from each subject who was eligible for the study. Placental and blood samples were handled in accordance with the principles of The Declaration of Helsinki at delivery by cesarean section or spontaneous labor. Each subject who were eligible for the study provided written informed consent and approval (approval no: 33-730 in 2011) for the use of human subjects was obtained from the institutional ethics committee. Infant characteristics such as gestational age, birth weight, birth length and head circumference were also recorded (Table 1).

Table 1
Demographic characteristics of mother-infant pairs.

	Mean ± S.D.*	*n* (%)
Mothers		
Age at birth of infant (years)	29.63 ± 4.94	
Education		
University		52 (53.6)
High School		35 (36.1)
Primary/secondary school		10 (10.3)
Occupation		
Working		67 (69.1)
Not working		30 (30.9)
Number of delivery by mothers		
1		58 (59.8)
2		29 (29.9)
3		7 (7.3)
4		1 (1.0)
Occupational exposure to lead		0 (0)
Infants		
Gender		
Male		48 (49.5)
Female		49 (50.5)
Gestational age (days)	272.12 ± 7.46	
Birth weights (kg)	3.32 ± 0.46	
Birth length (cm)	49.75 ± 1.97	
Head circumferences (cm)	35.28 ± 1.14	
Placental weights (g)	632.84 ± 152.98	
Placental lead (μg/kg)	8.35 ± 3.08	

* S.D.: standard deviation.

2.2. Determination of placental lead levels

To avoid external metal contamination, each placenta was placed in a plastic bag immediately after delivery. Each bag was marked with the subject's identification code, placed on ice in a portable refrigerator and transported to the freezer in the Ankara University Analytical Toxicology Laboratory. In order to prevent any contamination originating from maternal blood and mucus, all placental samples were washed prior to analysis. Each sample was washed with 0.01% Triton X-100 solution and then 3 times with distilled water. Six representative samples were cut from each placenta using titanium tools, excluding the chorionic plate and decidua basalis, for the lead analysis. Two samples were taken from the center, avoiding the umbilical cord insertion, and four samples were taken from within 3 cm of the outer placental margin between the central region and the periphery. Each sample was dried for 24 h at 75 °C and weighed. Samples were then dissolved in 10 ml of nitric acid in Teflon microwave tubes and digested at 800 W and 220 °C for 20 min in a CEM Mars Xpress microwave oven. Prior to analysis with dual atomic absorption spectrometry (AAS), the solutions were diluted with 25 ml deionized water in 50-ml polypropylene tubes. Lead levels were quantified using Varian AA 240 Z Zeeman Graphite Atomic Absorption Spectrometry (GFAAS) and the metal levels were given as μg/kg. Additionally, the AAS method was validated by evaluating certified reference materials (Seronorm™ Trace Elements Whole Blood Level-2; Ref Number: 201605) with known values.

2.3. Determination of the ALAD G177C SNP by the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method

Genomic DNA was isolated from 100-μl whole blood samples using a Qiagen QIAamp DNA Mini Kit, according to the manufacturer's instructions. The ALAD G177C polymorphism located on the