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## Aminolevulinic Acid Dehydratase Allelic Frequency and Lead Toxicity in Children Under-Five in a Former Used Lead-Acid Battery Area

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## Abstract

Polymorphisms in the *Aminolevulinic Acid Dehydratase (ALAD)* gene responsible for the *ALAD1* and *ALAD2* alleles have been implicated in susceptibility to lead toxicity. This study aimed to determine the allelic frequency of *ALAD2* among children living in Bogor District, Indonesia, and its association with blood lead levels (BLLs) and lead toxicity. A cross-sectional study involving 128 children was conducted during September-October 2019 in the former ULAB area in Cinangka Village. The *ALAD* polymorphism, BLLs, and hematological parameters were evaluated. Blood samples were taken for dried blood spotting on filter paper, blood film, and BLL measurement. The PCR amplification and sequencing of the genomic DNA revealed the presence of two forms of the *ALAD2* allele: 177C and 177T with a frequency of 0.05. Analysis of the correlation between the *ALAD2* allele, BLLs, and basophilic stippling revealed that *ALAD2* carriers had a five times higher risk of high BLLs, (OR = 5.359, p-value = 0.155) and had a slightly higher risk of exhibiting basophilic stippling (OR = 1.09, p-value = 1.000). Although not statistically significant, these findings suggested that the *ALAD* genotype may modify BLLs and lead to toxicity. The *ALAD2* allele (177T) is firstly reported in any population in the world.

**Keywords:** *Aminolevulinic acid dehydratase-2* allele, basophilic stippling, blood lead level, lead toxicity

## Introduction

Lead is one of the ten most dangerous chemicals for humans.<sup>1</sup> Long-term lead exposure was responsible for 1.06 million deaths and 24.4 million disability-adjusted life years (DALYs) worldwide in 2019.<sup>2</sup> The burden was the greatest in low- and middle-income countries (LMICs). Exposure to lead can occur through contaminated air, water, dust, food, or consumer products.<sup>1</sup> The amount of lead in blood and tissues, as well as the time course of the exposure, determine its toxicity.<sup>3</sup> Lead poisoning can cause various symptoms and signs, depending on the individual and the duration of lead exposure.<sup>4</sup> Early-life exposure to lead can re-program genes, resulting in altered gene expression and a higher risk of disease later in life.<sup>5</sup>

Young children are the most vulnerable to lead poisoning because they are more likely to put objects in their mouths, such as those containing lead paint, and absorb a greater proportion of lead.<sup>1,6</sup> Exposure at work is a common cause of lead poisoning in adults with certain occupations at high risk.<sup>7</sup> Poisoning with large doses of

lead damages the brain and central nervous system, resulting in coma, convulsions, and even death. Children who survive severe lead poisoning may develop intellectual disabilities as well as behavioral problems. At low levels of exposure that create no evident symptoms, lead has been shown to impair children's brain development, resulting in lower intelligence quotient (IQ), behavioral changes such as decreased attention span and increased antisocial behavior, and poor educational attainment. Symptoms of lead poisoning include anemia, hypertension, renal impairment, immunotoxicity, and toxicity to the reproductive organs. Lead is thought to have irreversible neurological and behavioral consequences.<sup>1</sup>

Lead poisoning can occur in the presence of erythrocyte inclusion objects.<sup>8</sup> Lead can cause erythrocyte hemolysis and inhibit the formation of hemoglobin. Lead also causes the enzyme glucose-6-phosphate dehydrogenase (G-6PD) deficiency and inhibits the enzyme pyrimidine-5'-nucleotidase. This causes a reduction in the life span of erythrocytes and increases the fragility of the erythrocyte membrane, thus reducing the number of ery-

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throcytes.<sup>9</sup> A deficiency in this enzyme is hereditarily characterized by basophilic stippling on erythrocytes. Previous studies have also shown that lead inhibits haem biosynthesis by inhibiting the enzyme's coproporphyrinogen,  $\delta$ -aminolevulinic acid dehydratase (*ALAD*), and ferrochelatase which reduces hemoglobin (Hb) levels in the blood.<sup>10,11</sup> The presence of lead in erythrocytes causes disruption of hemoglobin formation in erythrocytes. Furthermore, basophilic stippling in erythrocytes indicates a change in blood morphology, which shows that there has been disruption of haem synthesis in children due to lead exposure, although it did not cause anemia.<sup>12</sup> Only lead exposure levels above 50  $\mu\text{g}/\text{dL}$  for adults and 25-40  $\mu\text{g}/\text{dL}$  for children can cause basophilic stippling and microcytic or normocytic anemia.<sup>13</sup>

The Center for Disease Control (CDC) has set an upper limit for blood lead exposure for adults and children: 10  $\mu\text{g}/\text{dL}$  (10  $\mu\text{g}/100$  g) and 5  $\mu\text{g}/\text{dL}$ , respectively.<sup>14</sup> Elevated lead levels may be diagnosed by measuring the blood lead levels (BLLs).<sup>1</sup> Changes in levels or dense lines in the X-ray of children's bones can also be used to detect elevated lead levels.<sup>15</sup> Human hair might be employed as an alternative matrix to identify chronic lead exposure.<sup>16</sup> The  $\delta$ -aminolevulinic acid dehydratase (5-aminolevulinic acid dehydratase, EC 4.2.1.24, *ALADH*) is an enzyme of the haem biosynthetic pathway which catalyzes the condensation of two molecules of  $\delta$ -aminolevulinic acid to form a monopyrrole ring of porphobilinogen.<sup>17</sup> In humans, this enzyme is coded for by the *ALAD* gene located on chromosome 9q34.<sup>18</sup>

Previous studies have shown that *ALAD* gene polymorphisms and lead toxicity have a relative relationship with BLLs.<sup>19-21</sup> The *ALAD2* allele is associated with lead liver and kidney toxicity and damage to the hematopoietic system.<sup>22</sup> The prevalence of the *ALAD2* allele ranges from 0 to 20%, depending on the population. Generally, Caucasians have the highest frequency of the *ALAD2* allele, with approximately 18% of the Caucasian population being *ALAD1/2* heterozygotes and 1% being *ALAD2/2* homozygotes. In comparison, African and Asian populations have low frequencies of the *ALAD2* allele, with few or no *ALAD2* homozygotes being found in such populations.<sup>23</sup> In Indonesia, previous studies documented markedly different results regarding *ALAD* genotype frequencies. Firdausi, et al.,<sup>24</sup> reported the frequency of polymorphism in *ALAD* genes in the population of 60 elementary school students in Kalideres Subdistrict, West Jakarta Administrative City. They found an *ALAD1/1* frequency of 51.7% and *ALAD1/2* frequency of 48.3% and did not find any *ALAD2/2* homozygous individuals. Rujito, et al.,<sup>25</sup> found 91.8% of *ALAD1/1* and 8.2% *ALAD1/2* and did not find any *ALAD2/2* individuals among 67 gas station workers aged 20–60 years from seven gas stations in Banyumas

District. Shen, et al.,<sup>26</sup> studied the gene distribution of the *ALAD* isozyme phenotypes in 229 children aged 6–10 years who live within a community in which a large smelter is located; they found *ALAD1/1* (92%), *ALAD1/2* (8%), and *ALAD2/2* (0%). Diawara, et al.,<sup>27</sup> reported that among 251 children who lived next to smelting activities in Pueblo, Colorado, the United State of America, 94.4% of children had *ALAD1/1*, 5.6% had *ALAD1/2* polymorphisms, and none had *ALAD1/1* polymorphism.

Lead-acid batteries, also called car batteries, are rechargeable batteries in which lead plates in sulfuric acid are enclosed in a plastic shell. They are utilized in every country on the planet. The batteries can be recharged several times, but after several cycles, the lead plates degrade, and the battery's ability to store energy for any length of time is lost. When a lead-acid battery stops working, it becomes unusable and is designated as a used lead-acid battery (ULAB), which is a hazardous waste under the Basel Convention.<sup>28</sup>

Since 1978, Cinangka Village in Bogor District has been known as a center for ULAB recycling. This illegal anthropogenic activity aims to obtain lead blocks, resold as raw materials for various industries. Unfortunately, the recycling technology used is very simple. Thus, it poses a health risk and leads to environmental pollution. This illegal activity has been closed several times; however, people still secretly carry out these illegal activities behind their homes, where their children usually play. In 2014, in collaboration with Blacksmith, an American non-governmental organization (NGO), the Ministry of the Environment of the Republic of Indonesia carried out remediation of lead-contaminated land in Cinangka Village. However, these efforts have only recovered ~10% of the total lead-contaminated land in Cinangka Village. By the end of 2018, through the National Defense Council, the government again issued strict instructions regarding the prohibition of ULAB recycling in Cinangka Village. Thus, the authors of this study aimed to analyze the current condition of children in Cinangka Village after ULAB recycling was prohibited in the area. This study was done to determine the allelic frequency of *ALAD2* among children who lived near former ULAB recycling areas and chose its association with BLLs and lead toxicity. By knowing the *ALAD* gene profile in children, information about individual susceptibility to lead exposure can be obtained.

## Method

Cinangka Village is located at an altitude of 286 m above sea level and is in the Ciampea Subdistrict, Bogor District. This village has an area of 3.4 km<sup>2</sup> or ~11.10% of the area of the Ciampea Subdistrict. A total of 4,195 households are spread over ten hamlets.<sup>29</sup>

Based on information from local officials, of the 13,253 people living in Cinangka Village, 1,204 were children below five years old in 2021. The study sample consisted of 147 children who met the inclusion criteria: aged 1-5 years, with parents who lived in the study area for three years and agreed that their children were respondents. Samples were taken using a purposive sampling technique, and the study was carried out in four selected hamlets/*Rukun Warga* (RW) because high-intensity ULAB activities had previously occurred in these locations. Samples were taken by cluster: samples of 33 children were taken from hamlet 01, 71 from hamlet 03, 27 from hamlet 04, and 16 children from hamlet 06.

The sample size was calculated based on the Formula 1. The  $P_1$  is the proportion of the *ALAD* allele that had undergone polymorphism in the lead-exposed group. At the same time,  $P_2$  is the proportion of *ALAD* alleles that had undergone polymorphism in the lead-unexposed group. The value of the proportion of *ALAD* alleles in the lead-exposed and unexposed groups was obtained from the results of previous studies.<sup>19,30</sup> In the calculation, the level of significance ( $\alpha$ ) was set at 5% or  $Z_{1-\alpha/2}=1.96$ , and the power of the test ( $1-\beta$ ) was 80%. Therefore, the study required a minimum of 132 children to test the hypothesis of two population proportions. The sample sizes were increased by 10% to account for dropouts. The purposive sampling method recruited 147 children from different households. Of these, the blood of only 128 children was genetically analyzed, making this the total final sample for this study.

Three variables were evaluated: *ALAD* gene, BLLs, and basophilic stippling. Briefly, blood sampling was carried out on October 17-18, 2019, at the local village office. Integrated Service Post/*Pos Pelayanan Terpadu* (*Posyandu*) cadres helped arrange the arrival of the children with their mothers for blood collection. Each child's arm was cleaned with soap and water, dried with a tissue, wiped with an alcohol swab, and dried with gauze before drawing peripheral blood by phlebotomists. The blood lead level was measured using the LeadCare® II Portable Analyser. Rapid in situ diagnosis can be performed in less than three minutes with the LeadCare® II Portable Analyser. The analyzer expressed blood test results in  $\mu\text{g/dL}$ .

Deoxyribonucleic acid (DNA) was extracted from the dried blood spot using the Chelex method.<sup>31</sup> The DNA obtained was used as a template for polymerase chain reaction (PCR) amplification of the *ALAD* gene using the following oligomers: forward primers (5'-AGACAGACATTAGCTCAGTA-3' and reverse primers: 5'-GGCAAAGACCACGT CCATTC-3'),<sup>30</sup> under the following conditions: 45 cycles of initial denaturation at 94°C (5'), followed by denaturation at 94°C (30"), annealing at 54°C (30"), and extension at 72°C (1') fol-

$$n = \frac{\left\{ z_{1-\alpha/2} \sqrt{2 \bar{P} (1 - \bar{P})} + z_{1-\beta/2} \sqrt{P_1 (1 - P_1) + P_2 (1 - P_2)} \right\}}{(P_1 - P_2)^2}$$

Formula 1. Sample Size Calculation

lowed by a final extension at 72°C (4'). The amplicon obtained was verified on the agarose gel. If positive, the amplicons were prepared for DNA sequencing to identify the presence of the polymorphism at nucleotide 177 of the *ALAD* gene. The presence of the *ALAD* gene was indicated by the appearance of a band located parallel to the positive control. The normal allele PCR product was the *ALAD* gene allele, which is in the 917-bp band.

The polymerase chain reaction (PCR) products were purified using clean-up systems (PROMEGA Corporation, Madison, WI, USA) and Exonuclease I shrimp alkaline phosphatase (USB, Affymetrix, Cleveland, OH, USA). The purified amplicons were sequenced using an ABI Prism Dye Big Dye Terminator Cycle Sequencing Ready Kit (Applied Biosystem, Foster City, CA, USA) in a fluorescent DNA capillary electrophoresis sequencer (ABI 3130x1). The analysis was done at the Eijkman Institute (Jakarta, Indonesia). In case of ambiguity in sequencing reading, sequencing was repeated in both directions (forward and reverse primers).

The PCR method was validated by testing the reproducibility and specificity parameters of the PCR product. The PCR method met the reproducibility parameters if the same electropherogram was produced at 917-base pair (bp) from the PCR product of different DNA isolate samples. The PCR method met the specificity parameters if the nucleotide sequence of the PCR product at 917 bp matched the Genbank nucleotide sequence of the *ALAD* gene. Specificity was determined by sequencing the PCR product, and sequencing results were manually edited using the BioEdit program. The resulting nucleotide sequence was aligned with the *ALAD* gene sequence from GenBank using Clustal W, a part of the BioEdit program. Thin blood smears stained with Giemsa were identified for the presence of basophilic stippling using light microscopy (objective 1000x).

Chi-Square analysis was performed to evaluate the Hardy-Weinberg balance in the allelic distribution and identify the relationship between *ALAD* gene polymorphisms and the respondent's BLL. A p-value of less than 0.05 was considered statistically significant to verify the relationship between the *ALAD* gene polymorphism and the respondent's BLLs. The value of  $X^2$  indicated the Hardy-Weinberg balance compared to the critical p-value of 0.05 (df = n-1) in the Chi-square table (there is no



difference in the frequency of the *ALAD* gene allele in the population). If the value of  $X^2$  is less than critical value, there is a Hardy-Weinberg balance. Chi-square analysis was also used to evaluate the relationship between *ALAD* gene polymorphisms and the respondent's BLL and basophilic stippling.

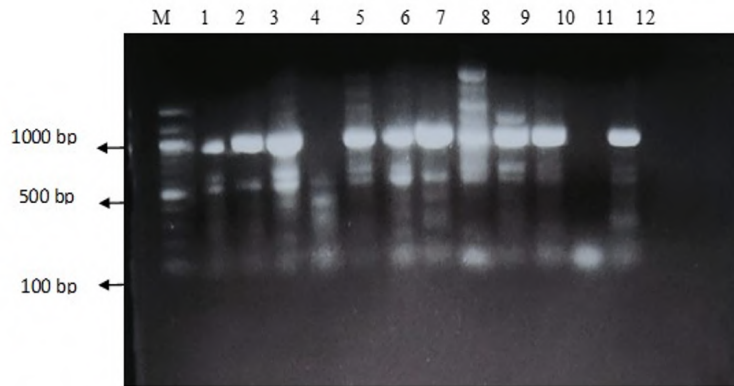
**Results**

Respondents were children aged 1–5 years whose parents had lived permanently in Cinangka Village for a minimum of three years. The children were selected due to the high susceptibility of lead exposure from the environment and the relatively low mobility compared to adults. A minimum exposure time of three years was used in study because environmental lead exposure occurs

chronically in small doses; thus, a long time is required to accumulate in the body.

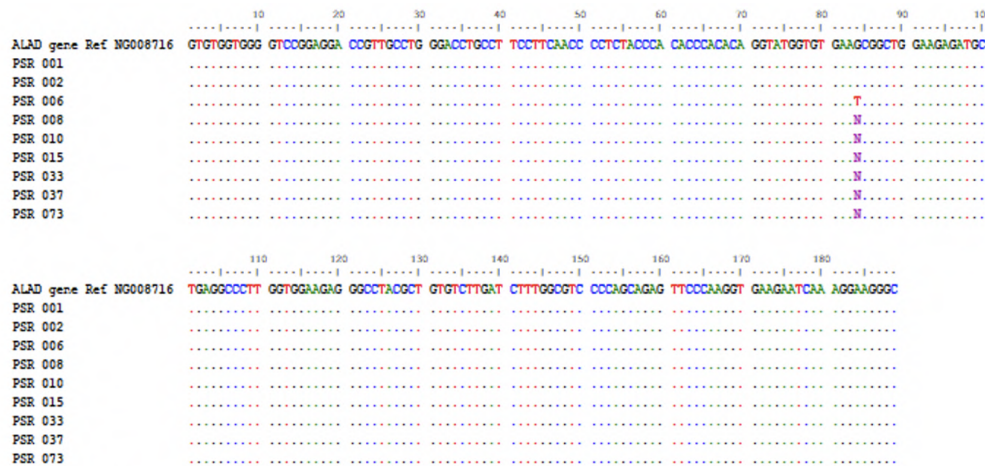
The PCR amplification of DNA samples extracted from 128 individuals yielded an amplicon of the expected size of 917 bp (Figure 1). The amplicon obtained was then purified and prepared for DNA sequencing.

The DNA sequencing results were aligned using the BioEdit Programme and compared with standard published sequences (Figure 2). Based on the polymorphism at nucleotide 177, the *ALAD2* allele was found in two forms (177C and 177T), in which the asparagine (N) residue is substituted by lysine (K) at codon 59 of the *ALAD* gene (Figure 3). The *ALAD* genotype frequency was *ALAD1/1* (90.6%), *ALAD1/2* (8.6%), and *ALAD2/2* (0.8%). The allelic frequencies of *ALAD1* and



Notes: Marker (M) 100 bp; Ladder (Lane 1-12); Sample No. 005, 006, 015, 017, 018, 021,037, 047, Negative Control, and Positive Control

Figure 1. PCR Products of *ALAD* Amplification with the 917-bp DNA Target



Notes: N in Sample PSR 008, 010, 015 = G/T; N in Sample PSR 033, 037, 073 = G/C

Figure 2. Alignment of DNA Sequencing Results of the *ALAD* Gene Fragment Encompassing Nucleotide 177

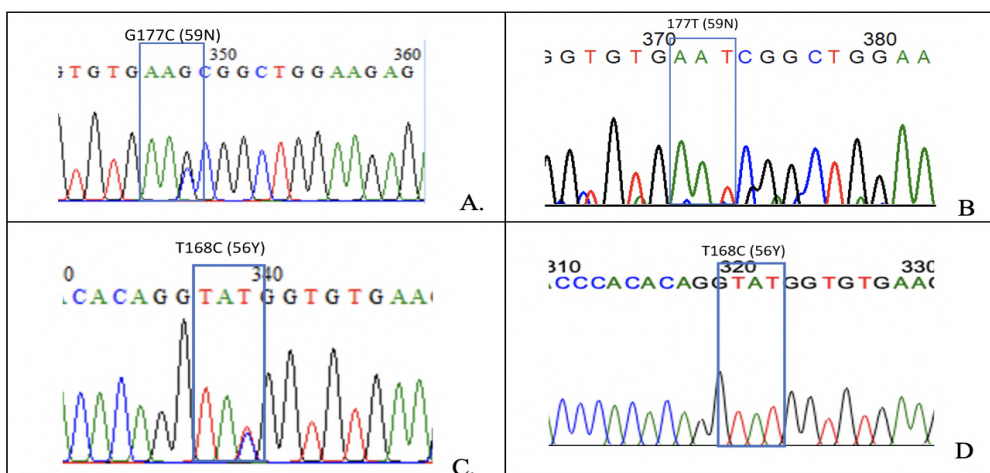


Figure 3. Electropherogram of the DNA Sequencing Results of the ALAD Gene Fragment Flanking Nucleotides 177 and 168

Table 1. Genotype Distribution of the Allelic Frequencies in the Study Population

ALAD Genotypes	n (Obs.)	Genotype Frequency	n (Exp.)	Allele Frequency
1-1 (GG)	116	0.906	115.33	ALAD1 = 0.95
1-2 (GC/GT)	11	0.086	12.34	ALAD2 = 0.05
2-2 (TT)	1	0.008	0.33	X <sup>2</sup> = 1.51
	128	1.000	128	df = 1; p-value > 0.05 (Critical value = 3.84)

Notes: ALAD = Aminolevulinic Acid Dehydratase, df = degree of freedom, Obs. = Observed, Exp. = Exposed

Table 2. Correlation of the Aminolevulinic Acid Dehydratase-2 Allele with Blood Lead Levels

ALAD Allele	Blood Lead Levels				Total	OR	95% CI	p-value	
	High		Low						
	n	%	n	%					
ALAD2	11	91.7	1	8.3	12	100	5.359	0.667-43.047	0.155
ALAD1	78	67.2	38	32.8	116	100			

Notes: ALAD = Aminolevulinic Acid Dehydratase, OR = Odds Ratio, CI = Confidence Interval

ALAD2 were 0.95 and 0.05, respectively (Table 1).

Comparison of the Chi-square results with the critical value showed that no difference existed in the frequency of the ALAD gene in the population over generations, which indicates that the Hardy-Weinberg equilibrium was established. In addition, a non-synonymous nucleotide substitution from C to T at base 168 of the ALAD gene was also found in 19 samples. This nucleotide substitution does not introduce any amino acid changes.

Analysis of the correlation between the ALAD2 allele and BLL using Chi-square revealed that subjects carrying the ALAD2 alleles had 5.3 times higher risk of having a

high BLL (Table 2). However, the results were not statistically significant (p-value = 0.155). Analysis of the correlation between the ALAD2 allele and basophilic stippling revealed that subjects carrying the ALAD2 allele had a slightly higher risk of experiencing basophilic stippling (OR = 1.09, p-value = 1.000) (Table 3).

### Discussion

The frequency distribution of the ALAD1 and ALAD2 alleles among different populations worldwide has been the subject of numerous investigations. These studies have used genotype and phenotype techniques and indicated that the ALAD2 frequency ranges from 0 to

Table 3. Correlation of the Aminolevulinic Acid Dehydratase-2 Allele with Basophilic Stippling

ALAD Allele	Basophilic Stippling						OR	95% CI	p-value
	Yes		No		Total				
	n	%	n	%	n	%			
ALAD2	3	25.0	9	75.0	12	100	1.0941	0.278–4.305	1.000
ALAD1	31	26.72	85	73.28	116	100			

Notes: ALAD = Aminolevulinic Acid Dehydratase, OR = Odds Ratio, CI = Confidence Interval

20%.<sup>18,23,32-34</sup> The *ALAD2* allelic frequency among different populations of Indonesia had been previously reported in several studies. Using the PCR-ARMS technique, Firdausi, *et al.*,<sup>24</sup> reported an *ALAD1/1* frequency of 51.7% and an *ALAD1/2* frequency of 48.3% without any *ALAD2/2* individuals in the population of 60 elementary school students in Kalideres Subdistrict, West Jakarta Administrative City. Another study reported an *ALAD1/1* frequency of 91.8% and an *ALAD1/2* frequency of 8.2% without any *ALAD2/2* individuals among 67 gas station workers in Banyumas District using the PCR-RFLP technique.<sup>25</sup> Using PCR DNA sequencing, this study found an *ALAD2* allelic frequency of 0.05 (same frequency as that observed in China,<sup>32</sup>), and surprisingly, a novel form of the *ALAD2* allele (177T) was discovered. This novel form has never been reported previously in any *ALAD* gene population study worldwide, and previous studies have only reported a G→C transversion for *ALAD2* allele polymorphism.<sup>19,35</sup> This finding might explain why a significantly different result was reported in previous studies that did not use PCR DNA sequencing. The DNA sequencing method can detect all nucleotide sequences in the target region of the *ALAD* gene so that previously undetected polymorphisms can be identified. Therefore, the frequency distribution of the *ALAD* gene in previous studies that have not included the T allele in their calculations can be revised. Based on the published *ALAD2* frequency among the Asian population, the finding of this study may be more reliable and valid.<sup>23</sup>

The *ALAD* G177C polymorphism yields two codominant alleles, *ALAD1* and *ALAD2*, and it has been implicated in the susceptibility to lead toxicity. The rarer *ALAD2* allele has been associated with high BLLs and has been thought to increase the risk of lead toxicity by generating a protein that binds lead more tightly than the *ALAD1* protein.<sup>23</sup> A G→C transversion occurs at position 177 of the *ALAD2* coding area resulting in the replacement of asparagine for lysine at amino acid 59. These two alleles give rise to three isozymes—1/1, 1/2, and 2/2—which all have identical functions but differ in charge. Asparagine is a positively charged amino acid, whereas lysine is neutral. Thus, *ALAD1/2* heterozygotes

create a more electronegative enzyme than *ALAD1* homozygotes, whereas *ALAD2* homozygotes produce a more electronegative enzyme than 1/2 heterozygotes.<sup>23</sup> Analysis of the correlation between the *ALAD2* allele and BLL in this study indicated the higher risk of having high BLL among subjects carrying the *ALAD2* allele. However, the result was not statistically significant. Similar findings have been reported previously in numerous population studies, particularly those living under higher lead exposure.<sup>22,26,35</sup> No association was observed between *ALAD2* and BLL, perhaps because the *ALAD* genotype might be a modifier of BLL only at high BLL concentrations.<sup>35</sup> Although this study did not find an association between the *ALAD2* allele and BLL, the mean BLL among *ALAD2* carriers was higher (29.41 µg/dL) than that among *ALAD1* carriers (17.14 µg/dL). This result proved that *ALAD2* could bind lead better than *ALAD1*.

Concerning the association between the *ALAD2* allele and basophilic stippling, this study found an unexpectedly different result in which *ALAD2* allele carriers only had a slightly higher risk of basophilic stippling in their erythrocytes. However, the result was not statistically significant. Previous studies have reported that basophilic stippling, particularly among children, is a clear sign of lead toxicity.<sup>36</sup> In this study, no association was observed between *ALAD2* and basophilic stippling. Similar to the relationship of *ALAD2* with BLL, this was possible because the association only occurs at high BLLs, which affected *ALAD2* activity. In addition, although it was found that children's BLL level in Cinangka Village exceeded the measurement limits of the LeadCare® II instrument (>65.0 µg/dL), because there were only two respondents, the available data were not representative enough to be analyzed statistically. Therefore, the insufficient number of samples was a limitation of this study, as did the absence of a control group. Further studies to explore the frequency of *ALAD* polymorphisms and its association with BLL and lead toxicity among different populations are required with an adequate number of samples.



## Conclusion

The allelic frequency of *ALAD2* among children living in Cinangka Village, Bogor District, is 0.05. Although not statistically significant, this study suggests that the *ALAD* genotype may modify BLLs and lead to toxicity. The *ALAD* genotyping method using PCR-sequencing can be applied for the detection of *ALAD* gene polymorphism in children with more precise results. Identifying the *ALAD* gene can be used as a screening tool for individual susceptibility to lead exposure. Further studies regarding the pathogenesis and pathophysiology of lead intoxication are needed to determine its relationship with *ALAD* gene polymorphisms.

## Abbreviations

*ALAD*: Aminolevulinic Acid Dehydratase; BLL: Blood Lead Level; DALYs: Disability-Adjusted Life Years; LMICs: Low- and Middle-Income Countries; IQ: Intelligence Quotient; Hb: Hemoglobin;  $\mu\text{g}/\text{dL}$ : microgram per deciliter; CDC: Centers for Disease Control and Prevention; ULAB: Used Lead-Acid Batteries; NGO: Non-Governmental Organization; RW: *Rukun Warga*; Posyandu: *Pos Pelayanan Terpadu*; DNA: Deoxyribonucleic Acid; PCR: Polymerase Chain Reaction.

## Ethics Approval and Consent to Participate

The study protocol was reviewed and approved by the ethical committee of the Faculty of Public Health, Universitas Indonesia (404/UN2.F10/PPM.00.02/2019). The parents were briefed about the protocol during the sampling process written informed consent before recruiting their children as participants of the study.

## Competing Interest

The author declares that there are no significant competing financial, professional, or personal interests might have affected the performance or presentation of the work described in this manuscript.

## Availability of Data and Materials

The data are fully available without restriction.

## Authors' Contribution

YI is involved in design studies, data analysis, compilation, and preparation of journal publications. HK and UFA are involved in design studies. DS and PBSA are involved in drafting publication journals. AS assisted with lead reagent procurement, facilitating LeadCare® II tools, and providing lead-related materials. AS<sub>t</sub> involved in technical assistance in sampling procedures. RR was involved in technical assistance in sampling procedures and genetic analysis. S assisted in basophilic stippling examination, DHP, LS, FKD assisted in genetic analysis.

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