Genetic polymorphism of \( \text{ALDH2} \) in Indonesia’s Minang ethnic

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Introduction

In the liver, alcohol is metabolized firstly to acetaldehyde by alcohol dehydrogenase (ADH), followed by oxidation to acetate by aldehyde dehydrogenase (ALDH) [1]. Some people cannot properly oxidize the acetaldehyde to acetate, and consequently, the toxic acetaldehyde accumulates in the blood. They experience facial flushing, dizziness, and hypertension when consuming food or beverages containing alcoholic substances. This condition is known as sensitivity to alcohol [2]. This condition is mainly caused by a deficiency of an isozyme of ALDH, namely ALDH2 [3]. The \( \text{ALDH2} \) is expressed in liver mitochondria, and the isozyme has the highest affinity to acetaldehyde [4]. The deficiency of \( \text{ALDH2} \) reduces the ability of \( \text{ALDH2} \) to oxidize acetaldehyde to acetate properly [5].

The polymorphism of \( \text{ALDH2} \) occurs because of the substitution of glutamate (GAA) into lysine (AAA) in 487 amino acid residue positions [4,6]. This substitution, from an acidic amino acid residue to a basic amino acid residue, should decrease the \( \text{ALDH2} \) enzyme activity. It was shown that alcohol-sensitive people have an atypical \( \text{ALDH2} \) gene and that atypical homozygote is more alcohol-sensitive than atypical heterozygote [7,8]. Atypical \( \text{ALDH2} \) gene is mainly found in Mongoloid ethnic (Japan and China), and rarely in Caucasians and Negroid ethnic [8,9].

The frequency of polymorphism in \( \text{ALDH2} \) is varied from one ethnicity to another [8].

ABSTRACT

Background: In some people, acetaldehyde, a toxic product from ethanol oxidation, cannot be oxidized to acetate. The excess of acetaldehyde could cause facial flushing, dizziness, and hypertension when they consume ethanol. This ethanol sensitivity is caused by a deficiency of \( \text{ALDH2} \).

Objective: This study aims to analyze and count the polymorphism frequency of the \( \text{ALDH2} \) gene in Indonesia’s Minang ethnic.

Methods: DNA samples were taken randomly from hair bulbous of 60 subjects (male and female, 3rd generation). A nested polymerase chain reaction was conducted to amplify the \( \text{ALDH2} \) in the samples. Afterward, restriction fragment length polymorphism (RFLP) was conducted to the amplicons using the \( \text{EcoRI} \) restriction enzyme. The measured parameters were the distribution of the wildtype, atypical homozygote, and heterozygote.

Results: Results showed that out of 60 subjects, 53.33% have an atypical homozygote gene (subjects prone to hypersensitive to alcohol), 28.33% have a heterozygote gene, and 18.33% have a wildtype gene. The frequency of the atypical alleles in Minang ethnic is 0.675.

Conclusion: The atypical \( \text{ALDH2} \) allele was much higher than normal \( \text{ALDH2} \) allele, in which most participants have atypical homozygote \( \text{ALDH2} \), suggesting the sample are sensitive to alcohol.

Keywords: \( \text{ALDH2} \), ethanol sensitivity, Minangkabau, polymorphism
Indonesia is an archipelago country with a very diverse ethnic. Each ethnic in Indonesia has its origin and history. This study was performed on samples from Minangkabau ethnic group. They originate from West Sumatra province, Sumatra island, Indonesia. They are distributed along the west coast of Sumatra, from northwest Aceh to southwest in Bengkulu, even in Malay-speaking countries such as Malaysia, Singapore, and Brunei. However, despite relatively cosmopolitan attitude and behavior, they tend to marry a partner from the same ethnic group, especially in Minangkabau in West Sumatra. From a genetic view, this tendency should be interesting. Therefore, this study aims to analyze the frequency of ALDH2 polymorphism in the Minang ethnic, one of the major Malay ethnic groups from Sumatra, the western part of Indonesia.

Methods
Subjects

Hair bulbous was used as a sample [10]. Six hair bulbous were collected from each respondent from 60 volunteers. All of them were Minang descendants for three generations.

DNA extraction

Hair bulbs was collected in a 1.5 mL microcentrifuge tube. Then 50 μL of DNA extraction buffer was added to the microtube. The microtubes were incubated at 55°C for one hour and then proceeded with 95°C incubation for 10 minutes. The extracted DNA was stored at -20°C and be used for PCR I.

Nested polymerase chain reaction

Materials for PCR were Master Mix 2× (Promega), two forward primers, a reverse primer, and sterile ddH₂O. Primers consisted of: Forward 1 (F1): CAAATTACAGGGTCAACTGC; Forward 2 (F2): TATGATGTGTTTGGAGCCCAG; Reverse (R): TTAAGTTTTGACACTCACGC. The PCR condition was 94°C, 5 minutes followed by (94°C for 30 seconds, 53°C for 30 seconds, 72°C for 30 seconds) for 30 cycles, 72°C for 7 minutes, and hold at 4°C. Amplicons from PCR I were used as a DNA template for PCR II, with the same condition as PCR I. The amplicons were then used for restriction fragment length polymorphism.

Restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP) was conducted using the EcoRI restriction enzyme (Roche). Polyacrylamide-gel electrophoresis (PAGE) was conducted using acrylamide (Merck), bisacrylamide, TAE buffer 10× pH 8.4, TEMED, ammonium persulphate, loading dye (consists of bromphenol blue and xylene cyanol), DNA molecular weight marker, ethidium bromide, and sterile ddH₂O. RFLP was conducted by mixing amplicons from PCR II 15 μL, buffer enzyme for EcoRI 2 μL, BSA 2 μL, and EcoRI enzyme 1 μL. The mixture was incubated at 37°C for 2 hours and used for SDS-PAGE electrophoresis.

Data analysis

The allele frequency of ALDH2 was determined using this formula:

\[ \text{allele frequency} = \frac{\Sigma \text{atypical allele}}{\Sigma \text{whole allele}} \]

An atypical allele of ALDH2 is an allele consisting of a mutated ALDH2. Thus, a wildtype consists of two normal alleles, whereas heterozygote and homozygote consist of one atypical allele and two atypical alleles, respectively.

Results

The result of electrophoresis analysis on the restriction pattern on the PCR product of ALDH2 is presented in Figure 1. The results showed two bands (80 bp and 100 bp) and only one band, either 80 or 100 bp. The second lane showed an 80 bp band, whereas the third and sixth have a 100 bp band. The fourth and fifth lanes showed both 80 and 100 bp. The seventh lane was non-template control (NTC). The restriction patterns were obtained after incubating each amplicon with EcoRI, with 5'-GAATTCC-3' as the restriction site.
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PCR analysis were made on samples from 60 persons. Figure 2 shows the bar plot of the distribution of the ALDH2 gene of the Minang ethnic. The data showed that wildtype, atypical heterozygote, and atypical homozygote ALDH2 gene were present in Minang ethnic. The atypical ALDH2 allele was much higher than the normal ALDH2 allele, in which most participants have atypical homozygote ALDH2, suggesting the sample majority are sensitive to alcohol. The result showed that the frequency of the atypical allele was 0.675, and the normal allele was 0.325.

**Discussion**

Alcohol-sensitive individuals have a mutation in which glutamic acid (an acidic amino acid residue) is changed to lysine (a basic amino acid residue), causing the change of protein structure and function. At DNA level, this mutation could be revealed by restriction pattern analysis. In the normal (wildtype) gene, EcoRI cut the 100 bp amplicons into 80 and 20 bp. In this study, the 80 bp band sequence was shown in the second lane in the gel, while the 20 bp band was not seen in the electrophoresis because it is too small to be detected. On the other hand, when the EcoRI restriction enzyme meets a mutated ALDH2 gene from alcohol-sensitive individuals, the enzyme cannot cut the sequence because there is no restriction site. Therefore, the amplicons remained intact. The intact band of 100 bp was found in the third lane in the gel. This lane shows the atypical homozygote, which means both alleles are mutated.

Some samples show 100 bp and 80 bp as in the fourth and the fifth lane (Figure 1). These individuals have an atypical heterozygote gene. One allele has the wild-type gene, and the other has the mutated gene. The mutant enzyme has a lower affinity to aldehyde. Consequently, there would be an aldehyde, mainly acetaldehyde accumulation in tissue, especially the lower tissue.

The previous study showed that atypical ALDH2 genotype frequency in Minang ethnic (81.66%) is higher than the general population of Indonesian
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...atypical $\text{ALDH2}$ genotype frequency (30%) [11]. Interestingly, the diversity of the $\text{ALDH2}$ gene in one ethnic is very different from the general Indonesian [11]. It can also be said that the frequency of atypical $\text{ALDH2}$ allele in western Indonesia is relatively high. The diversity of ethnicities in Indonesia can influence the difference in $\text{ALDH2}$ allele frequency.

It is proposed that the population of Sumatra island originated from East Asia, and traveled to Vietnam, then to the Malay Peninsula, where it spreads towards Sumatra, Java, and Borneo [12]. This proposed migration theory corresponds with our finding that the frequency of atypical $\text{ALDH2}$ is high, the same as oriental ethnics. The origin of the Sumatra population could be the explanation for the high number of atypical $\text{ALDH2}$ genotypes. These theories suggest that Sumatra and generally the western part of Indonesia share common ancestry with oriental ethnics. It remains to be seen whether Java and Borneo people share the same tendency as West Sumatran people regarding $\text{ALDH2}$ allele frequency, considering that they all have the same ancestry. It is also interesting to study the tendency of $\text{ALDH2}$ allele frequency in other Indonesia parts that do not share the same ancestry (the eastern part of Indonesia).

Although only using a small sample, this study suggests that most Minangkabau population are sensitive to alcohol. This trait might protect an individual from alcohol abuse and alcoholism [13,14]. Since most Minangkabau population are Muslims, alcoholism is not much of a problem. However, they often consume fermented food which contains ethanol. The implication is that the Minang people might be susceptible to diseases related to alcohol sensitivity. A study has shown that atypical $\text{ALDH2}$ alleles risk colorectal cancer [15]. A meta-analysis also suggests that atypical $\text{ALDH2}$ allele may be associated with increased risk of coronary artery disease and myocardial infarction [16]. A study also indicates a relation between atypical ALDH2 allele and elevated blood pressure [17].

**Conclusion**

Although only using a small sample, most Minangkabau populations are sensitive to alcohol, and the frequency of atypical $\text{ALDH2}$ in Minangkabau is higher than in general population of Indonesian. Further studies regarding the correlation between the $\text{ALDH2}$ gene and the risk of alcohol-related diseases (alcohol abuse and alcoholism) as well as colorectal cancer and myocardial infarction are required.

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Author contributions
AHS and ID performed the experiment, AHS wrote the manuscript, M provided the samples, SIW and RP conceptualize, develop the methodology, and provided expertise and feedback.

Declaration of interest
The authors do not have any conflict of interest.

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