Journal of Tropical Biodiversity and Biotechnology
Volume 10, Issue 03 (2025): jtbb16566
DOI: 10.22146/jtbb.16566

Research Article

Frequency of *ALDH2* Gene Polymorphism (rs671) in Dayak, Javanese, Minahasan, and Papuan Ethnic Populations

Jerry Ferry Langkun[1], Rully Adi Nugroho[1], Ferry Ferdy Karwur[2]*
1)Faculty of Biology, Satya Wacana Christian University, Jl. Diponegoro 52-60, Salatiga, Jawa Tengah, 50711, Indonesia
2)Faculty of Health Sciences, Satya Wacana Christian University, Jl. Kartini 11A, Salatiga, Jawa Tengah, 50711, Indonesia
* Corresponding author, email: ferry.karwur@uksw.edu

Keywords:
Alcohol
Aldehyde dehydrogenase-2
Allele frequency
Acetaldehyde
Genotype frequency
South East Asia
Submitted:
24 September 2024
Accepted:
29 March 2025
Published:
04 August 2025
Editors:
Ardaning Nuriliani
Annisaa Widyasari

ABSTRACT

The rs671 single nucleotide polymorphism (SNP) in the *ALDH2* gene results in ALDH2 enzyme inactivation, impairing acetaldehyde detoxification. Individuals carrying the *ALDH2*2* allele are at increased risk for alcohol-related diseases. The rs671 polymorphism is common in East Asia, likely originating from the Han-Chinese ethnic group. Studies in Indonesia report high variability in the frequency of the *ALDH2*2* allele, ranging from 15.5 % to 72.6 %, raising questions about why its prevalence in Indonesia exceeds that of China. This study aimed to determine the genotypic and allele frequencies of rs671 among Indonesians from different ethnic backgrounds, including the Dayak, Javanese, Minahasan, and highland Papuan groups. SNP rs671 was detected using the TaqMan SNP genotyping assay on the QuantStudio 5 real-time PCR system. In the Minahasan group, the genotypic frequencies were 97.8 % typical homozygous (*ALDH2*1/*1*), 2.2 % heterozygous (*ALDH2*1/*2*), and 0 % atypical homozygous (*ALDH2*2/*2*), with an allele frequency of 1.1 % for *ALDH2*2*. In contrast, the Dayak, Javanese, and highland Papuan groups exhibited 100 % typical homozygotes (ALDH2*1/*1), with no rs671 variants detected. These results suggest a lower prevalence of rs671 SNPs in the Minahasan group and the absence of variants in Javanese and highland Papuan populations, differing from prior reports. The absence of rs671 in highland Papuans indicates that this polymorphism likely emerged and spread after the divergence of East Asian and Papuan populations. Further studies are needed to elucidate the evolutionary and migratory history of rs671 in Southeast Asia.

How to cite:
Langkun, J.F., Nugroho, R.A. & Karwur, F.F., 2025. Frequency of *ALDH2* Gene Polymorphism (rs671) in Dayak, Javanese, Minahasan, and Papuan Ethnic Populations. *Journal of Tropical Biodiversity and Biotechnology*, 10(3), jtbb16566. doi: 10.22146/jtbb.16566

INTRODUCTION

Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are the two main enzymes involved in alcohol metabolism (Edenberg 2007). Most of the alcohol metabolism processes occur in liver cells, where the ADH enzyme oxidises ethanol to acetaldehyde, and the ALDH enzyme oxidises acetaldehyde to acetate (Edenberg 2007). The ALDH enzyme exists in several isozymes. The ALDH2 enzyme is commonly found in mitochondria and is one of the isozymes of ALDH which has a very important role in the detoxification of acetaldehyde in the human body (Cederbaum 2012). ALDH2 has a very high affinity for acetaldehyde, as a toxic metabolite of ethanol so it is very efficient at converting acetaldehyde into acetic acid (Cederbaum 2012).

Deficiency in ALDH2 enzyme activity inhibits acetaldehyde metabolism, resulting in its accumulation and increased concentration of acetaldehyde in the blood after alcohol consumption (Lee et al. 2019). ALDH2 enzyme deficiency increases sensitivity to alcohol, and the accumulation of acetaldehyde in the body may cause discomfort such as facial flushing, lightheadedness, rapid heartbeat, motion sickness and vomiting, and headache (Agrawal & Bierut 2012; Cederbaum 2012). Hypersensitivity to alcohol, with the symptom of facial flushing after drinking alcohol found in East Asia populations, was the first phenotypic symptom known to be associated with deficiency of ALDH2 enzyme activity (Harada et al. 1980). The results of previous studies indicate that ALDH2 enzyme deficiency is associated with polymorphisms in the gene coding for this enzyme. Research into the relationship between ALDH2 enzyme deficiency and ALDH2 gene polymorphism began with the discovery of a substitution of glutamic acid with lysine in the inactive ALDH2 enzyme (Yoshida et al. 1984), and the identification of single-point mutations in ALDH2 DNA clones (Hsu et al. 1985; Yoshida et al. 1985). The research on *ALDH2* gene polymorphism progressed with the discovery of the structure of the *ALDH2* gene by Hsu et al. (1988).

The *ALDH2* gene is located on chromosome 12 at q24.2 (12q24.2), with a gene structure composed of 43099bp (13 exons), coding for 517 amino acids to produce 56kDa *ALDH2* protein (Matsumoto 2019). The mutation occurs in exon 12, where guanine (G) is replaced by adenine (A), resulting in a change in gene expression from the amino acid Glu to Lys (Yoshida et al. 1984; Hsu et al. 1985). Substitution of guanine with adenine causes a change in the typical allele of *ALDH2*1* (wild type) to atypical allele of *ALDH2*2* (variant) resulting in an enzyme that is dominant negative, i.e. the activity of the enzyme decreases drastically in heterozygotes and is inactive in homozygotes (Crabb et al. 1989).

The presence of the *ALDH2*2* allele has a protective effect again excessive alcohol consumption and alcohol dependence (Edenberg & McClintick 2018) thereby reducing the risk of developing related diseases. Discomforts such as flushing, nausea, chest palpitations, and drunkenness make individuals carrying the *ALDH2*2* allele more likely to avoid consuming alcohol. On the other hand, the dominant social and cultural factors of alcohol consumption tend to eliminate this preventive effect (Ting et al. 2015). This explains the result of previous studies revealed an association between rs671 and diseases related to alcohol consumption (Han et al. 2019; Kim et al. 2020; Yokoyama et al. 2021). In that case, the presence of the *ALDH2*2* allele will increase the risk of developing diseases related to alcohol consumption, such as liver diseases (Yokoyama et al. 2017), stroke (Sun et al. 2017), hypertension (Han et al. 2019; Yoo et al. 2020; Kim et al. 2021), and cancer (Suo et al. 2019; Ugai et al. 2019). Other studies have shown an association between rs671 and hyperuricemia and gout (Sakiyama et al. 2016; Zhang et al. 2018).

The allele of rs671 is common in East Asia populations but almost absent in Europe, Africa, and among American Indians (Goedde et al. 1992; Li

et al. 2009; Katsarou et al. 2017). In China, Japan, and Korea, the prevalence of the *ALDH2*2* allele ranges from 16 to 40.9 %, while in Tibet, Mongolia, Thailand, Vietnam, Laos, Malaysia, it is around 1-10 %, and in Taiwan-Aboriginal and Filipino, only around 0.6-3 % (Goedde et al. 1992; Chen et al. 1997; Luo et al. 2009; Luczak et al. 2017). Research data consistently shows that the highest frequency is found among the Han ethnicity in Central China, the coasts of South China and the coasts of East China (Zhong et al. 2018; Millwood et al. 2019). Li et al. (2009) and Luo et al. (2009) concluded that the *ALDH2*2* allele originated in the Han-Chinese ethnic group which spread throughout China and around Asia following the migration of the Han ethnic group.

Data on the prevalence of rs671 among ethnic groups in Indonesia are still very limited. Research in Jakarta showed that the frequency of the *ALDH2*2* allele was quite high, at 15.5 % (Wanandi 2002). Other research on ethnic Papuans, East Nusa Tenggara, and Javanese populations shows a high prevalence of *ALDH2*2*: 28.2, 40.2, and 72.6 %, respectively (Nugroho 2018; Suhartini et al. 2019; Busyra et al. 2021).

Data on active alcohol drinkers in several regions in Indonesia is quite high, namely 11–16 % for men aged ≥10 years (RISKESDAS 2018), 13.4–31.5 % for men aged ≥15 years (Suhardi 2011), and tends to increase from year to year (World Health Organization 2018). Therefore, the high prevalence of rs671 in Indonesia needs to be studied further because the presence of the *ALDH2*2* allele increases the risk of diseases related to alcohol consumption. In addition, it raises the question whether the prevalence of the *ALDH2*2* allele in Indonesia is higher compared to China, which is believed to be the origin of the *ALDH2*2* allele. It is necessary to study the distribution pattern of the *ALDH2*2* alleles in Indonesia, considering that geographically Indonesia is divided into three areas, namely the western part which in the past was a unit with the Asian continent (*Sundaland*), the eastern part which in the past was a unit with the Australian continent (*Sahul-land*), and the *Wallacea* area which is in the middle (Bellwood 2017). In addition, Indonesia has two major racial groups: the Austronesian-speaking Mongolids and the Papuans, who speak Tras-Papuan (non-Austronesian) languages (Diamond & Bellwood 2003).

A study on the frequency of rs671 in Indonesia, based on ethnic background and geographic location, compared to the distribution of the *ALDH2*2* allele in East Asia, Southeast Asia and Oceania is important to be carried out. The aim of this study was to determine the genotype and allele frequencies of rs671 in the Dayak, Javanese, Minahasan, representing the Mongoloid, and highland Papuan representing the Austromelanesoid.

MATERIALS AND METHODS

Participants

This study examined a total of 289 participants consisting of four population groups: Dayak, Javanese, Minahasan, and highland Papuan ethnic backgrounds. The participant's ethnic background was obtained through the participant's personal confession of parent's origin (In the Minahasa and highland Papuan, the family name can be used as an initial guide). For the Dayak and highland Papuan samples, tracking of ethnic background was carried out down to their grandparents. The Dayak, Javanese, and Minahasan ethnic groups represent the descendants of the Austronesian-speaking Mongolid race, while the highland Papuan ethnic groups represent the descendants of the Papuan race (Diamond & Bellwood 2003). Based on geographical location, the Javanese and Dayak ethnic groups represent populations living in an area that was formerly part of Asia continent (*Sundaland*), while the highland Papuan represents people who live in the area that used to join the Australian

continent as the Sahul Shelf (*Sahul-land*) (Bellwood 2017). The Minahasan ethnic group represents the *Wallacea* area which is located between *Sundaland* and the *Sahul-land* (Bellwood 2017).

The 95 Javanese participants came from the village of Tegalrejo Salatiga, Central Java. The Minahasan participants consisted of 96 people from North Sulawesi (51 people came from villages in the Belang Health Center, Southeast Minahasa Regency and 45 people came from Tara-Tara 1 Village, Tomohon City). The 50 Papuan participants were taken from students currently studying in Salatiga, originating from several sub-ethnic groups of the highland Papuan (Dani, Lani, Amungme, Ngalum, Nduga, Damal, Yali, Mee, Muyu, Ketengban, Moni, Komoro) originally from the Central Highlands of West Papua, Indonesia. The 48 Dayak ethnic participants came from Bengkayang Regency in the working area of Bengkayang Hospital, Samalantan Health Center, Bengkayang Health Center, Sungai Betung Health Center, Lumar Health Center, Sanggau Ledo Health Center, and Jagoi Babang Health Center. Participants were not differentiated by sex because previous research showed that gender had no effect on the prevalence of rs671 (Wu et al. 2021).

All participants were more than 18 years old and given informed consent to be signed as proof of willingness to participate voluntarily. The research protocol was approved by the Research Ethics Committee of the Faculty of Medicine and Health Sciences, Satya Wacana Christian University.

Blood sampling and DNA extraction

All participants had their blood taken approximately 3 mL of blood samples by certified nurse staff. The blood sample was put into a vacutainer tube with a purple lid containing EDTA buffer. Samples were stored in an ice box with ice gel cooling during transportation from the sampling location to the laboratory. In the laboratory, blood samples were stored in a freezer at -20°C. Total genomic DNA was extracted from whole blood samples using the Genomic DNA Mini Kit: Blood/Cultured Cell reagent (Geneaid, #cat:GB100/300) according to the manufacturer's protocol.

DNA quality test

Good DNA quality is the basis for determining and preparing samples for real-time PCR testing. The rigidity test of the DNA was done using the agarose gel (0.8 %) electrophoresis method. DNA electrophoresis agarose gels were stained with ethidium bromide (1 µg mL^{-1}) and visualised using a UV transilluminator (CleverView, Clever Scientific) (Sambrook & Russell 2001). The DNA concentration was calculated based on the absorbance at a wavelength of 260 nm according to the Beer-Lambert formula. DNA purity was calculated based on the absorbance ratio at a wavelength of 260 nm and 280 nm with a good DNA purity value between 1.7–2.0 (Bruijns et al. 2022). DNA absorbance was measured using a Shimadzu 1900i UV-Vis spectrophotometer.

SNP Genotyping Assays (rs671)

The *ALDH2* gene polymorphism (rs671) was detected using the TaqMan SNP genotyping assay method with the QuantStudio 5 Applied Biosystems real-time PCR and Taqman SNP assay kit (Thermofisher Scientific, C_11703892_10) (Huang et al. 2017; Chien et al. 2019; Koyanagi et al. 2021; Tokiya et al. 2024). Reactions were performed in a 20 µL reaction mix consisting of 5 µL DNA sample (10 ng), 10 µL of 1× TaqMan GTXpress Master Mix (Applied Biosystems, Foster City, CA), 1 µL of 20 × TaqMan SNP Genotyping Assays rs671 Reagent (Thermofisher Scientific, C_11703892_10: #4362691), and 4 µL of Nuclease-Free Water. The rs671 test procedure referred to the reagent kit manufacturer protocol with real-time PCR settings

as follows: pre-read (25 °C, 30 seconds), hold (95 °C, 20 seconds), 40× cycle [denaturation (95 °C, 3 seconds), annealing/extention (60 °C, 30 seconds)], and post-read (25 °C, 30 seconds).

Data Analysis

Data from SNP genotyping assay real-time PCR were processed using the QuantStudio Design & Analysis v1.5 application and presented in the form of spreadsheet data, while the quality of the test results can be seen based on the amplification plot graphs and allelic discrimination plot graphs. Amplification plot graphs were made using QuantStudio Design & Analysis v1.5 software and allelic discrimination plot graphs were made using TaqMan Genotyper v1.4 software. TaqMan Genotyper software was also used to calculate the chi -square and *P*-value according to the Hardy-Weinberg Equilibrium (HWE) principle. Chi-square and P-value calculations for other research data were also conducted to assess conformity with the HWE principle. The $P>0.05$ means the proportion of sample genotype frequencies according to the HWE principle (Graffelman 2020).

The rs671 polymorphism was presented in two categories: first based on genotypic frequencies, namely homozygous typical (*ALDH2*1/*1*), heterozygous (*ALDH2*1/*2*) and homozygous atypical (*ALDH2*2/*2*), and second based on allele frequencies, namely typical allele (*ALDH2*1*) and atypical allele (*ALDH2*2*) (Zhong et al. 2018). Descriptive analysis was used to compare the rs671 genotype and allele frequencies with distribution data in East Asia, Southeast Asia, and Oceania as well as with data on several ethnic groups in Indonesia in previous studies.

RESULTS

The SNP genotyping assay was carried out in stages according to the capacity of the real-time PCR tool (96 samples), starting with Javanese ethnic sample and continuing with Minahasan, Papuan, and Dayak ethnic samples. Negative control (Negative Control Template, NTC) was included in each test series. Heterozygous genotype samples found in the Minahasa ethnic group were included as positive controls for the heterozygous genotype in the next sample test, namely highland Papuan and Dayak ethnic groups, serving as quality control to see the consistency of testing on each batch. Four samples of Minahasan ethnicity from Taratara Village were undetermined. All Dayak, Javanese and Papuan ethnic samples were successfully read in the test. Thus, a total of 285 samples could be analysed further (95 Javanese, 92 Minahasan, 50 highland Papuan, and 48 Dayak ethnic groups).

The amplification plot curve of the test results for each ethnicity is shown in Figure 1. The Y axis on the amplification plot curve is the fluorescence signal and the X axis is the number of PCR cycles. The blue colour curve shows the fluorescence signal amplification for the typical *ALDH2*1* allele (C_11703892_10-G), while the red colour represents the atypical *ALDH2*2* allele (C_11703892_10-A). The allelic discrimination plot, shown in Figure 2, illustrates the quality of genotype group separation for each ethnicity. The blue plot shows typical homozygous (G/G), the green plot shows heterozygous (G/A), the red plot shows atypical homozygotes (A/A), and the light blue plot shows the negative control template (NTC).

The amplification curve in Figure 1 shows that all Javanese, Dayak, and highland Papuan ethnic samples were amplified only on the typical *ALDH2*1* allele. In the Minahasan ethnic group, 90 samples were amplified only on the typical *ALDH2*1* allele, while two samples were amplified for both the atypical *ALDH2*1* allele and *ALDH2*2* typical allele, respectively. Separation of genotypes is more clearly seen in the Allelic discrimination plot curve (Figure 2), where the plot of all Javanese, Dayak, and highland Papuan ethnic samples

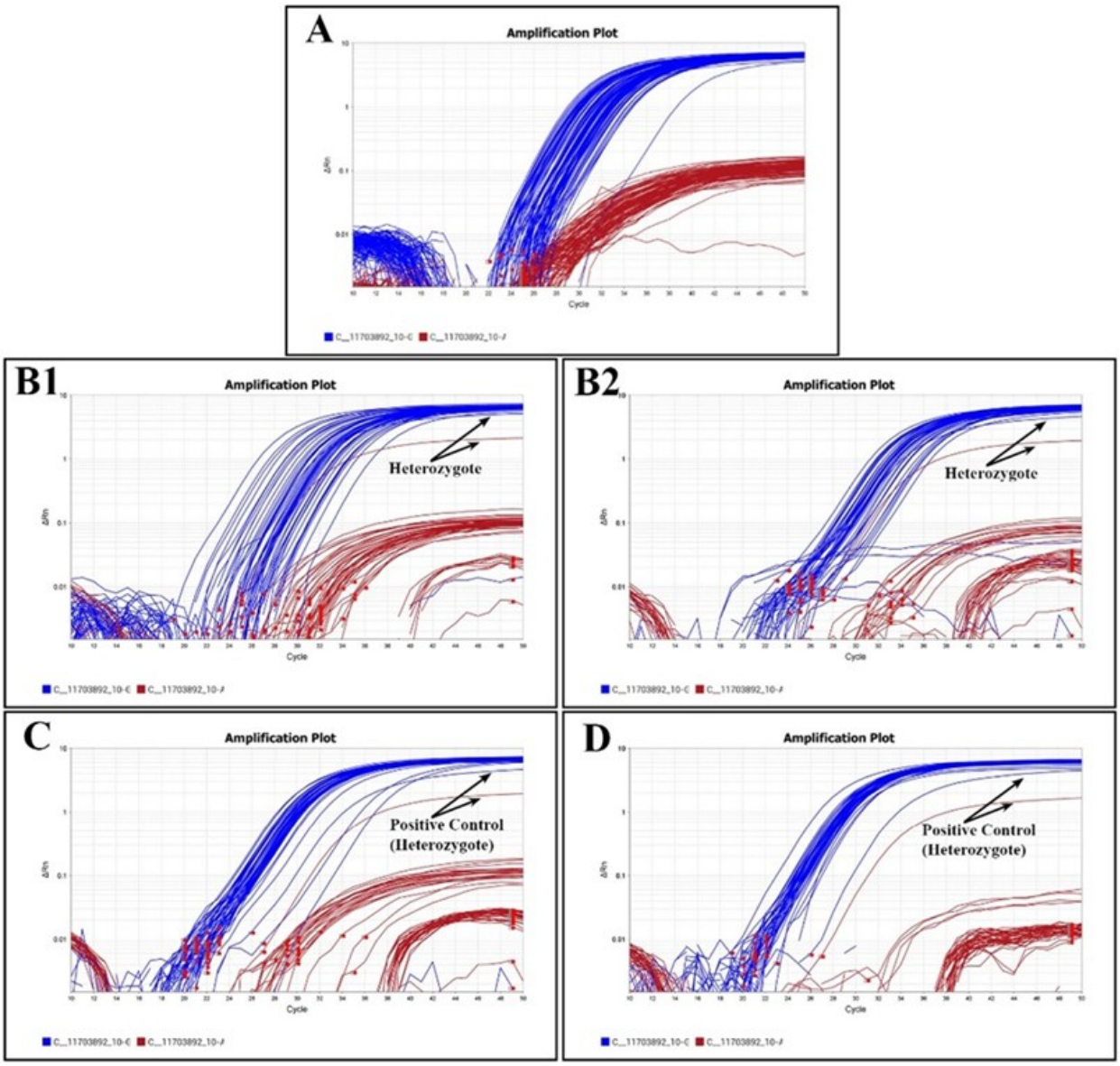

Figure 1. Amplification plot curve of Javanese (A), Minahasan (B1 and B2), Highland Papuan (C), and Dayak (D) samples.

(blue plot) clusters on the Y axis as the G/G group (*ALDH2*1/*1*). In the Minahasa ethnic group, 90 samples are clustered on the Y-axis side (*ALDH2*1/*1*) and two samples (green plot) are clustered separately on the diagonal line as the G/A group (*ALDH2*1/*2*). The heterozygous positive control appearance of the Minahasan ethnic samples included in the Dayak and highland Papuan ethnic testing indicates the consistency of the testing.

The rs671 frequency data are presented based on typical homozygous (*ALD2*1/*1*), heterozygous (*ALD2*1/*2*), and atypical homozygous (*ALD2*2/*2*) allele variations, as well as two variations of the [typical allele (*1) and atypical alleles (*2)]. In Table 1, the genotypic frequencies of the Dayak, Javanese, and highland Papuan ethnic groups show the same results: all individuals (100 %) were homozygous *ALDH2*1/*1*, with no atypical *ALDH2*2/*2* alleles observed. The genotypic frequency of the Minahasan ethnic group shows that there were 90 individuals (97.8 %) homozygous *ALDH2*1/*1*, two individuals (2.2 %) heterozygous *ALDH2*1/*2*, and none homozygous *ALDH2*2/*2*. The allele frequencies for the Minahasan ethnic group were 98.9 % typical *ALDH2*1* alleles and 1.1 % atypical *ALDH2*2* alleles, while the Dayak, Javanese and highland Papuan ethnic groups show the same values, i.e. 100 % typical *ALDH2*1* alleles. The frequency distribution of *ALDH2* polymorphism genotypes in the Minahasan ethnic group com-

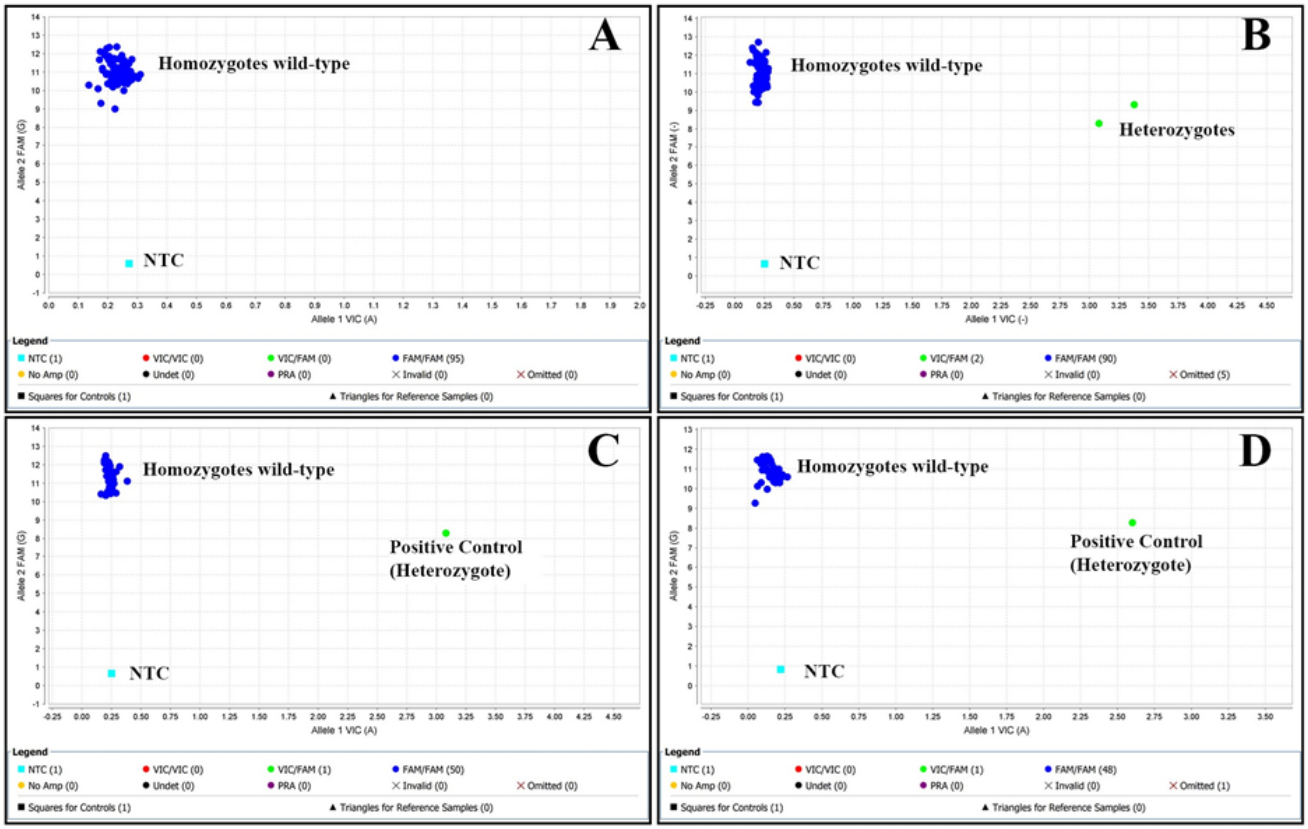

Figure 2. Allelic discrimination plots of Javanese (A), Minahasan (B), Highland Papuan (C), and Dayak (D) samples.

Table 1. *ALDH2* genotype and allele frequencies of Dayak, Javanese, Minahasan, and Highland Papuan ethnic group in Indonesia.

Ethnic Groups	N	*ALDH2* Genotype, n (%)			χ^2	*P-HWE*	*Frequency ALDH2* Alel	
		**1/*1*	**1/*2*	**2/*2*			**1*	**2*
Dayak	48	48 (100)	0 (0)	0 (0)	0	1	100	0
Javanese	95	95 (100)	0 (0)	0 (0)	0	1	100	0
Minahasan	92	90 (97.8)	2 (2.2)	0 (0)	0.011	0.916	98.9	1.1
Highland Papuan	50	50 (100)	0 (0)	0 (0)	0	1	100	0

plies with the Hardy-Weinberg equilibrium principle (c^2=0.011, p=0.916).

The results of this study indicate that rs671 was found only in the Minahasan ethnic group, at a very low frequency, and was absent in the Dayak, Javanese, and highland Papuan ethnic groups. Based on racial background, rs671 was not found in the Melanesoid population in Papua or in the Astronesian-speaking Mongolid populations in Kalimantan and Java, but was found in the Astronesian-speaking Mongoloid population in Minahasa.

DISCUSSION

Studies on the global distribution pattern of rs67, based on geographical location and ethnic background, state that rs671 originated in China and spread throughout China and around Asia following the Han-Chinese ethnic migration (Oota et al. 2004; Li et al. 2009; Luo et al. 2009; Zhang et al. 2021). There is very little research on the prevalence of rs671 in Archipelagic Southeast Asia, including Indonesia. Research data on rs671 in Indonesia are quite diverse and some are even contradictory.

Data on the distribution of rs671 in East Asia, Southeast Asia and Oceania based on geographical location and ethnic background are presented in

Table 2. In this study, rs671 was not found on the islands of Java and Kalimantan, islands that were formerly a unitary unit with Mainland Southeast Asia called *Sundaland* (Bellwood 2017). This result is consistent with the findings of Ariyono (2016), but contrary to the results of the research by Suhartini et al. (2019), who reported an *ALDH2*2* allele frequency reaching 72.6 %. The results of this study also show that rs671 was not found in highland Papua, a region geographically adjacent to the Australian continent, formerly part of the Sahul-land. Our result of the absence of the rs671 variant among highland Papuan is in accordance with the results of Goedde et al. (1992) and Li et al. (2009) in Papua New Guinea (PNG) and reject the result reported by Nugroho (2018) who found that the *ALDH2*2* allele frequency was 28.2 %, which was too high and biased from overall patterns of rs671 among Papuan, and resemble the frequency in East Asia Population.

Based on this research and distribution data in Mainland Southeast Asia, the spread of rs671 to southern China is thought to have stopped at the tip of the Mainland Southeast Asia peninsula (Malaysia) and did not reach the islands of Kalimantan and Java. The absence of rs671 in the highland Papuan ethnic group is allegedly due to its geographical location which is remote and

Table 2. *ALDH2*2* allele frequencies in populations in the regions of East Asia, Southeast Asia and Oceania.

Area	Country/ethnicity	*ALDH2*2* (%)	Reference
East Asia	Chinese-Han	17.0 – 40.9	Li et al. 2009
	Chinese-Han	11.0 – 30.9	Luo et al. 2009
	Chinese-Han	17.2	Wu et al. 2021
	Chinese-Hakka	28.1	Zhong et al. 2018
	China	13.0 – 29.0	Millwood et al. 2019
	China	29.4	Wu et al. 2017
	Chinese in America	30.0	Luczak et al. 2017
	Japan	11.1 – 34.1	Li et al. 2009
	Japan	26.2	Yokoyama et al. 2021
	South Korean	21.6	Li et al. 2009
	Korean in America	18.5	Luczak et al. 2017
	Korean	15.8	Kim et al. 2021
	Taiwanese (Han)	30.1	Huang et al. 2017
	Taiwanese-Aborigin	0.9–3.3	Chen et al. 1997
Mainland Southeast Asia	Vietnam	12.2–17.9	Li et al. 2009
	Kamboja	14.0	Oota et al. 2004
	Laos	0.0–10.7	Li et al. 2009
	Thailand	10	Goedde et al. 1992
	Thailand	7.9	Assanangkornchai et al. 2003
	Malaysia	3.4	Goedde et al. 1992
Archipelagic Southeast Asia	Phillipine	0.6	Goedde et al. 1992
	Indonesia:		
	- Dayak	0	This study
	Javanese	0	This study
	Javanese	0	Ariyono 2016
	Javanese	72.6	Suhartini et al. 2019
	Minahasa	1.1	This study
	Papua	0	This study
	Papua	28.2	Nugroho 2018
	NTT	40.2	Busyra et al. 2021
	Indonesia	15.5	Wanandi 2002
Oceania	Papua New Guinea	0.004	Goedde et al. 1992
	Papua New Guinea	0	Li et al. 2009
	Samoa	0	Li et al. 2009
	Polynesia	0	Chambers et al. 2002

far from East Asia and has a background of the Austromelanesoid race which chronologically came to Indonesia long before the arrival of the Mongoloid race (Bellwood 2017). This assumption is supported by the results of previous studies which did not find rs671 in Papua New Guinea (Li et al. 2009) and Australian-Aborigines (Goedde et al. 1992).

The Minahasan ethnic group, residing on the northern peninsula of Sulawesi, an island in the *Wallacea* area (Bellwood 2017), was the only ethnic group in this study who had rs671 with an *ALDH2*2* allele frequency of 1.1 %. This low prevalence of rs671 is similar to findings in Taiwanese-Aborigines (0.9-3.6 %) and Philippines (0.6 %) (Goedde et al. 1992; Chen et al. 1997), but differs significantly from the results of (Busyra et al. 2021) in East Nusa Tenggara (located in the *Wallacea* area) with an *ALDH2*2* allele frequency of 40.2 % (Table 2). The prevalence of rs671 in Minahasa may follow the Out of Taiwan migration theory, which states that the origins of the Austronesian-speaking Mongolid race originated from the island of Formosa (Taiwan) and migrated to the Philippines, Kalimantan, and Sulawesi, spreaded throughout the archipelago to Madagascar and Oceania (Bellwood 2017). The low frequency of the *ALDH2*2* allele in Minahasa, contrasted with its absence in Kalimantan, indicates that the results of this study need to be interpreted with caution.

The low prevalence of rs671 in Minahasa and its absence in Kalimantan and Java in this study, when compared to similar findings among some indigenous groups in Laos, such as the Katu, Phunoi, and Lamet ethnicities (Li et al. 2009), and in Oceania (Goedde et al. 1992; Chambers et al. 2002), could indicate that the spread of rs671 did not occur during the early migration of the Mongoloid race to Archipelagic Southeast Asia around 4,300 BC, or even during the migration of the Austronesian-speaking Mongoloid race between 2,500 and 500 BC (Bellwood 2017). This is also in accordance with Luo et al. (2009) who states that rs671 was brought to Mainland Southeast Asia during the warring state period (476–221 BC) by the Pai-Yuei tribe (indigenous people on the coast of Southeast China), some of whom migrated to North Vietnam when their country was controlled by other tribes, and to North Thailand during the Han dynasty (156–87 BC). Oota et al. (2004) state that rs671 is classified as a young SNP, presumably appearing for the first time in populations in East Asia.

As mentioned above, there are several research findings in Indonesia that are very different compared to our results and to the global distribution pattern of rs671. This is inconsistent with the hypothesis that rs671 originates from Han-Chinese. Table 3 shows the prevalence of rs671 in Indonesia compared to several data in East Asia which so far has the high distribution and prevalence of rs671. The studies by Suhartini et al. (2019), Nugroho (2018) and Busyra et al. (2021) in addition to being contrary to the data of this study, had higher frequency of the *ALDH2*2* allele (28.2–72.6 %) compared to global data, even higher than China (11.0–40.9 %), Japan (11.1–26.2 %) and Korea (15.8–21.6 %). Moreover, the genotypic proportions of the three studies deviated from the Hardy-Weinberg Equilibrium (HWE) principle ($P<0.05$). Data show that the genotypic frequencies in each of these studies include a higher proportion of atypical homozygotes (*ALDH2*2/*2*) than heterozygotes (*ALDH2*1/*2*). Different data is shown by the results of other studies in the East Asia, where the frequency of heterozygous genotypes is much greater than atypical homozygous according to the HWE principle (Hosking et al. 2004).

The results of Wanandi's (2002) research comply with the HWE principle and has a genotypic frequency proportion similar to research data in East Asia with typical homozygous (70 %), heterozygous (29 %), and atypical homozygous (1 %) genotypic frequencies. However, the research data is not ac-

Tabel. 3. The genotype and allele frequency of rs671 in Indonesia compared to East Asia.

Area/ Ethnicity	N	Genotype (%)			Alelle (%)	*HWE*[a]		Reference
		**1/*1*	**1/*2*	**2/*2*	**2*	c^2	*P*	
China	7,966	52.0	39.7	8.3	28.1	2.9	0.090	Zhong et al. 2018
	364	76.7	20.6	2.7	13.1	3.1	0.079	Han et al. 2019
	1,235	49.1	43.0	7.9	29.4	1.5	0.218	Wu et al. 2017
Japan	1,260	50.3	40.6	9.1	31.9	0.7	0.411	Koyanagi et al. 2017
	1,016	53.8	39.9	6.3	26.2	0.9	0.338	Yokoyama et al. 2021
Korea	214	65.9	31.3	2.8	18.5	0.3	0.558	Luczak et al. 2017
	23,313	70.8	26.7	2,5	15.8	0.0001	0.994	Kim et al. 2021
Indonesia	100	70.0	29.0	1.0	15.5	1.2	0.284	Wanandi 2002
Minahasa	92	97.8	2.2	0	1.1	0.011	0.916	This study
Java	199	27.1	0.5	72.4	72.6	194.0	< 0.001	Suhartini et al. 2019
Papua Highland	39	64.1	15.4	20.5	28.2	15.0	< 0.001	Nugroho 2018
NTT	51	52.9	13.7	33.3	40.2	26.4	< 0.001	Busyra et al. 2021

[a] Chi-square test for conformity with HWE where P > 0.05 means the distribution of genotypes is in accordance with the HWE principle.

companied by ethnic background.

Differences in results may occur due to differences in location, samples, and methods used. In Indonesia, SNP assay has so far used the Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) method (Wanandi 2002; Nugroho 2018; Suhartini et al. 2019; Busyra et al. 2021). The PCR-RFLP workflow is quite long, starting with PCR followed by restriction enzymes and data visualization by electrophoresis. In contrast, the Taqman probe genotyping assay qPCR method used in this study is concise, fast, and real-time data. Osaki et al. (2011) showed that the Taqman probe qPCR method was as accurate as sequencing, while the PCR-RFLP method, in certain cases, resulted in a higher frequency of atypical genotypes than expected. For a small number of SNPs and large samples, the TaqMan genotyping assay using real-time PCR is preferred due to its high throughput, time efficiency, and cost-effective, highly accurate and precise comparable to Sanger sequencing (Shen et al. 2009; Osaki et al. 2011; Zhang et al 2015).

One of the limitations of this research is that the sampling area for the Javanese is concentrated in one area at the sub-village level. Therefore, research with a broader geographic area and larger sample size is needed. Group representation in the population (sub-ethnicity) and a clear history of heredity are needed to answer whether it is true that the frequency of the *ALDH2*2* allele is not found in certain ethnicities or there is a possibility of different results in other population groups even though it is the same ethnicity. In addition, the number of ethnic groups also needs to be added to get a more complete picture of the pattern of rs671 distribution in Indonesia and in Archipelagic Southeast Asia. From a health perspective, the finding of the *ALDH2*2* allele in the Minahasan ethnic group is significant for further research, as this region has the highest number of active alcohol drinkers in Indonesia (Suhardi 2011; RISKESDAS 2018).

CONCLUSION

The *ALDH2* gene polymorphism (rs671) was found in the Minahasan ethnic group at a very low *ALDH2*2* allele frequency (1.1 %) but was not found in the Javanese, Dayak, or highland Papuan ethnic groups. The results of this study contrast with those of several other studies in Indonesia. The frequency of rs671 in the Dayak, Javanese, Minahasan, and highland Papuan ethnic groups in this study is consistent with the distribution pattern of rs671 observed in China, Mainland Southeast Asia, Archipelagic Southeast Asia, and

Oceania. This study confirms the hypothesis that the *ALDH2*2* allele is characteristic of East Asia.

ACKNOWLEDGEMENT

Acknowledgments are extended to all participants who kindly agreed to provide blood samples as a source of genotyping in this study, as well as to the Regional Government of Tomohon City and Bengkayang. All blood samples in this study were supplied by F.F.K. We are also grateful to the individuals involved in blood collection at the following locations: Tomohon (Treesia Sujana, Arwin Nusawakan, Meyga Lakukua); Belang (Maria Yunita Lengkong, Claudia Lembong); Salatiga (dr. Adjar, Venti Agustina), Papuan students in Salatiga (Debby Enock, Monica Yocku); Bengkayang (Joko, Juliani, Devi, Seli Marseli, Septiana).

AUTHORS' CONTRIBUTION

J.F.L., RAN, and FFK designed this study. JFL was responsible for all phases of the research. JFL and FFK conducted the laboratory work. JFL, RAN, and FFK analyzed the data. JFL, RAN, and FFK wrote the manuscript. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Agrawal, A. & Bierut, L., 2012. Identifying genetic variation for alcohol dependence. *Alcohol Research: Current Reviews*, 34(3), pp.274–281.

Ariyono, A., 2016. *Analisis genetik aldehid dehidrogenase 2 (ALDH2) pada non peminum alkohol suku Jawa*. Universitas Gajah Mada

Assanangkornchai, S. et al., 2003. Aldehyde dehydrogenase 2 genotypes, alcohol flushing symptoms and drinking patterns in Thai men. *Psychiatry Research*, 118(1), pp.9–17. doi: 10.1016/S0165-1781(03)00043-X.

Bellwood, P.S., 2017. *First islanders: prehistory and human migration in Island Southeast Asia*. New York: Wiley.

Bruijns, B. et al., 2022. Performance of spectrophotometric and fluorometric DNA quantification methods. *Analytica*, 3(3), pp.371–384. doi: 10.3390/analytica3030025.

Busyra, B. et al., 2021. Genetic polymorphism of ALDH2 linkage to kidney function status of East Nusa Tenggara alcohol drinkers and cigarette smokers. *Journal of Community Empowerment for Health*, 4(2), pp.46–52. doi: 10.22146/jcoemph.61559.

Cederbaum, A.I., 2012. Alcohol Metabolism. *Clinics in Liver Disease*, 16(4), pp.667–685. doi: 10.1016/j.cld.2012.08.002.

Chambers, G.K. et al., 2002. The genetics of alcoholism in Polynesians: Alcohol and aldehyde dehydrogenase genotypes in young men. *Alcohol, Clinical and Experimental Research*, 26(7), pp.949-955 doi: 10.1097/01.ALC.0000021145.47616.38.

Chen, W.J. et al., 1997. Alcohol dehydrogenase and aldehyde dehydrogenase genotypes and alcoholism among Taiwanese Aborigines. *Biological Psychiatry*, 41, pp.703–709.

Chien, H-T. et al., 2019. Alcohol-metabolizing Enzymes' Gene Polymorphisms and Susceptibility to Multiple Head and Neck Cancers. *Cancer prevention research (Philadelphia, Pa.)*, 12(4), pp.247-254. doi: 10.1158/1940-6207.CAPR-18-0449

Crabb, D.W. et al., 1989. Genotypes for aldehyde dehydrogenase deficiency and alcohol sensitivity - the inactive ALDH2*2 allele is dominant. *Journal of Clinical Investigation*, 83, pp.314–316.

Diamond, J. & Bellwood, P., 2003. Farmers and their languages: The first expansions. *Science*, 300(5619), pp.597–603. doi: 10.1126/science.1078208.

Edenberg, H.J., 2007. The genetics of alcohol metabolism role of alcohol dehydrogenase and aldehyde dehydrogenase variants. *Alcohol Research & Health*, 30, pp.5–13.

Edenberg, H.J. & McClintick, J.N., 2018. Alcohol dehydrogenases, aldehyde dehydrogenases, and alcohol use disorders: A critical review. *Alcohol, Clinical and Experimental Research*, 42(12), pp.2281–2297. doi: 10.1111/acer.13904.

Goedde, H.W. et al., 1992. Distribution of ADH2 and ALDH2 genotypes in different populations. *Human Genetics*, 88, pp.344–346.

Graffelman, J., 2020. Statistical tests for the Hardy–Weinberg Equilibrium. In *Wiley StatsRef: Statistics Reference Online*. Wiley Online Library. doi: 10.1002/9781118445112.stat08274.

Han, S. et al., 2019. Acetaldehyde dehydrogenase 2 rs671 polymorphism affects hypertension susceptibility and lipid profiles in a Chinese population. *DNA and Cell Biology*, 38(9), pp.962-968. doi: 10.1089/dna.2019.4647.

Harada, S. et al., 1980. Liver alcohol dehydrogenase and aldehyde dehydrogenase in the Japanese: isozyme variation and its possible role in alcohol intoxication. *The American Journal of Human Genetics*, 32, pp.8–15.

Hosking, L. et al., 2004. Detection of genotyping errors by Hardy-Weinberg equilibrium testing. *European Journal of Human Genetics*, 12(5), pp.395–399. doi: 10.1038/sj.ejhg.5201164.

Hsu, L.C., Bendel, R.E. & Yoshida, A., 1988. Genomic structure of the human mitochondrial aldehyde dehydrogenase gene. *Genomics*, 2, pp.57–65.

Hsu, L.C. et al., 1985. Cloning of cDNAs for human aldehyde dehydrogenases 1 and 2. *Proceedings of the National Academy of Sciences*, 82(11), pp.3771–3775.

Huang, C.C. et al., 2017. Investigating the association between alcohol and risk of head and neck cancer in Taiwan. *Scientific Reports*, 7(1), 9701. doi: 10.1038/s41598-017-08802-4.

Katsarou, M.S. et al., 2017. Effect of single-nucleotide polymorphisms in ADH1B, ADH4, ADH1C, OPRM1, DRD2, BDNF, and ALDH2 genes on alcohol dependence in a Caucasian population. *Pharmacology Research & Perspectives*, 5(4), e00326. doi: 10.1002/prp2.326.

Kim, H.Y. et al., 2020. Effect modification of acetaldehyde dehydrogenase 2 rs671 polymorphism on the association between alcohol intake and blood pressure: The dong-gu study. *Journal of Korean Medical Science*, 35(9), e14. doi: 10.3346/jkms.2020.35.e14.

Kim, S.S., Park, S. & Jin, H.S., 2021. Interaction between ALDH2 rs671 and life habits affects the risk of hypertension in Koreans: A STROBE observational study. *Medicine*, 100(28), e26664. doi: 10.1097/MD.0000000000026664.

Koyanagi, Y.N. et. al., 2017. Development of a prediction model and estimation of cumulative risk for upper aerodigestive tract cancer on the basis of the aldehyde dehydrogenase 2 genotype and alcohol consumption in a Japanese population. *European Journal of Cancer Prevention*, 26(1), pp.38–47. doi: 10.1097/CEJ.0000000000000222.

Lee, Y.J. et al., 2019. The association between alcohol metabolism and genetic variants of ADH1A, SRPRB, and PGM1 in Korea. *Alcohol*, 79, pp.137–145. doi: 10.1016/j.alcohol.2019.03.004.

Li, H. et al., 2009. Refined geographic distribution of the oriental ALDH2* 504Lys (nee 487Lys) variant. *Annals of Human Genetics*, 73(3), pp.335–345. doi: 10.1111/j.1469-1809.2009.00517.x.

Luczak, S.E., Liang, T. & Wall, T.L., 2017. Age of drinking initiation as a risk factor for alcohol use disorder symptoms is moderated by ALDH2*2 and ethnicity. *Journal of Psychopathology and Clinical Science*, 41(10), pp.1738–1744. doi: 10.1111/acer.13469.

Luo, H.R. et al., 2009. Origin and dispersal of atypical aldehyde dehydrogenase ALDH2*487Lys. *Gene*, 435(1-2), pp.96–103. doi: 10.1016/j.gene.2008.12.021.

Matsumoto, A., 2019. The bidirectional effect of defective ALDH2 polymorphism and disease prevention. In *Aldehyde Dehydrogenases*. Advances in Experimental Medicine and Biology, vol 1993. Springer, Singapore. doi: 10.1007/978-981-13-6260-6_4.

Millwood, I.Y. et al., 2019. Conventional and genetic evidence on alcohol and vascular disease aetiology: a prospective study of 500000 men and women in China. *The Lancet*, 393(10183), pp.1831–1842. doi: 10.1016/S0140-6736(18)31772-0.

Nugroho, N.A., 2018. *Hubungan antara polimorfisme gen ALDH2 dengan kebiasaan minum alkohol pada mahasiswa papua di Yogyakarta*. Universitas Gadjah Mada.

Oota, H. et al., 2004. The evolution and population genetics of the ALDH2 locus: Random genetic drift, selection, and low levels of recombination. *Annals of Human Genetics*, 68(2), pp.93–109. doi: 10.1046/j.1529-8817.2003.00060.x.

Osaki, R. et al., 2011. Accuracy of genotyping using the TaqMan PCR assay for single nucleotide polymorphisms responsible for thiopurine sensitivity in Japanese patients with inflammatory bowel disease. *Experimental and Therapeutic Medicine*, 2(5), pp.783–786. doi: 10.3892/etm.2011.287.

RISKESDAS, 2018. *Riset Kesehatan Dasar*. Jakarta: BALITBANGKES KEMENKES RI.

Sakiyama, M. et al., 2016. Identification of rs671, a common variant of ALDH2, as a gout susceptibility locus. *Scientific Reports*, 6, 25360. doi: 10.1038/srep25360.

Sambrook, J.F. & Russell, D.W., 2001. *Molecular cloning, a laboratory manual. third edition*, New York: Cold Spring Harbor Laboratory Press.

Shen, G.Q., 2009. The TaqMan Method for SNP Genotyping. In *Single Nucleotide Polymorphisms*. Methods in Molecular Biology™, vol 578. Humana Press, Totowa, NJ. doi: 10.1007/978-1-60327-411-1_19

Suhardi, 2011. Preferensi peminum alkohol di Indonesia menurut RISKESDAS 2007. *Buletin Penelitian Kesehatan*, 39(4), pp.154–164.

Suhartini et al., 2019. Analysis of effect of aldehyde dehydrogenase 2 (ALDH2) gene polymorphism on liver function status of alcohol drinkers in Indonesia. *AIP Conference Proceedings*, 2099, 020025. doi: 10.1063/1.5098430.

Sun, S. et al., 2017. Genetic polymorphisms in the ALDH2 gene and the risk of ischemic stroke in a Chinese han population. *Oncotarget*, 8(60), pp.101936-101943. doi: 10.18632/oncotarget.21803

Suo, C. et al., 2019. Alcohol intake interacts with functional genetic polymorphisms of aldehyde dehydrogenase (ALDH2) and alcohol dehydrogenase (ADH) to increase esophageal squamous cell cancer risk. *Journal of Thoracic Oncology*, 14(4), pp.712–725. doi: 10.1016/j.jtho.2018.12.023.

Ting, T.T. et al., 2015. Effects of genetic variants of ADH1B and ALDH2 and social network on continued alcohol drinking among young adolescents in Taiwan. *Drug Alcohol Depend*, 147, pp.38–45. doi: 10.1016/j.drugalcdep.2014.12.014.

Tokiya M. et al., 2024. Asian flush gene variant increases mild cognitive impairment risk: a cross-sectional study of the Yoshinogari Brain MRI Checkup Cohort. *Environmental Health and Preventive Medicine*, 29, pp.55. doi: 10.1265/ehpm.24-00214

Ugai, T. et al., 2019. The functional ALDH2 polymorphism is associated with breast cancer risk: A pooled analysis from the breast cancer association consortium. *Molecular Genetics & Genomic Medicine*, 7(6), e707. doi: 10.1002/mgg3.707.

Wanandi, S., 2002. Genetic polymorphism of aldehyde dehydrogenase-2 (ALDH-2) Distribution of genetic polymorphism of aldehyde dehydrogenase-2 (ALDH2) in Indonesian subjects. *Medical Journal of Indonesia*, 11(3), pp.135–142. doi: 10.13181/mji.v11i3.62.

World Health Organization, 2018. *Global status report on alcohol and health 2018*.

Wu, J. et al., 2021. Analysis of gender-specific associations between aldehyde dehydrogenase 2 (ALDH2) rs671 genetic polymorphisms and serum uric acid levels in Han Chinese. *Annals of Translational Medicine*, 9(9), pp.772–772. doi: 10.21037/atm-20-7113.

Wu, Y. et al., 2017. Positive association between ALDH2 rs671 polymorphism and essential hypertension: A case-control study and meta-analysis. *PLoS ONE*, 12(5), e0177023. doi: 10.1371/journal.pone.0177023.

Yokoyama, A. et al., 2017. Slow-metabolizing ADH1B and inactive heterozygous ALDH2 increase vulnerability to fatty liver in Japanese men with alcohol dependence. *Journal of Gastroenterology*, 53(5), pp.660–669. doi: 10.1007/s00535-017-1402-6.

Yokoyama, A. et al., 2021. Combinations of alcohol-induced flushing with genetic polymorphisms of alcohol and aldehyde dehydrogenases and the risk of alcohol dependence in Japanese men and women. *PLoS ONE*, 16 (7), e0255276. doi: 10.1371/journal.pone.0255276.

Yoo, M.G. et al., 2020. Association between the incidence of hypertension and alcohol consumption pattern and the alcohol flushing response: A 12-year follow-up study. *Alcohol*, 89, pp.43–48. doi: 10.1016/j.alcohol.2020.07.001.

Yoshida, A., Huang, I.-Y. & Ikawa, M., 1984. Molecular abnormality of an inactive aldehyde dehydrogenase variant commonly found in Orientals. *Proceedings of the National Academy of Sciences*, 81, pp.258–261.

Yoshida, A. et al., 1985. Molecular abnormality and cDNA cloning of human aldehyde dehydrogenases. *Alcohol*, 2, pp.103–106.

Zhang, D. et al., 2018. The polymorphism rs671 at ALDH2 associated with serum uric acid levels in Chinese Han males: A genome-wide association study. *Gene*, 651, pp.62–69. doi: 10.1016/j.gene.2018.01.064.

Zhang, L. et al., 2015. Genotyping on ALDH2: Comparison of Four Different Technologies. *PloS ONE*, 10(3), e0122745. doi: 10.1371/journal.pone.0122745

Zhang, X., Sun, A. & Ge, J., 2021. Origin and spread of the ALDH2 Glu504Lys allele. *Phenomics*, 1(5), pp.222–228. doi: 10.1007/s43657-021-00017-y.

Zhong, Z. et al., 2018. Genetic polymorphisms of the mitochondrial aldehyde dehydrogenase ALDH2 gene in a large ethnic Hakka population in southern china. *Medical Science Monitor*, 24, pp.2038–2044. doi: 10.12659/MSM.906606