Golden Sun-Rise
International Journal of Multidisciplinary on Science and Management
ISSN: 3048-5037/ Volume 3 Issue 3 Jul-Sept 2026 / Page No: 86-94
Paper Id: IJMSM-V3I3P107 / Doi:10.71141/30485037/V3I3P107

Original Research Article

Detection of the Human ALDH2 Gene Polymorphism Related to Alcohol Metabolism Capacity Using Allele-Specific PCR: A Pilot Study in Vietnam

Mai Van Phong[1], Vu Phuong Thao[2], Nguyen Thao Vy[3], Bui Hai Trung[4], Nguyen Phu Xuan Nghi[5], Thai Hong Quynh Chi[6]

[1]Polytechnic College of – Engineering and Technology,Vietnam.

[2]Hanoi - Amsterdam High School for the Gifted, Hanoi, Vietnam.

[3]Le Hong Phong High School for the Gifted, Ho Chi Minh City, Vietnam.

[4]HUS High School for Gifted Students, Vietnam National University, Hanoi, Vietnam.

[5]Tran Dai Nghia High School for the Gifted, Ho Chi Minh City, Vietnam.

[6]Hanoi - Amsterdam High School for the Gifted, Hanoi, Vietnam.

Received: 02 August 2026 Revised: 04 August 2026 Accepted: 06 August 2026 Published: 09 August 2026

Abstract - *Human alcohol metabolism is heavily dictated by aldehyde dehydrogenase 2 (ALDH2), an essential mitochondrial enzyme responsible for oxidizing toxic acetaldehyde into non-toxic acetate. A single nucleotide polymorphism (SNP) in exon 12 of the ALDH2 gene (G-to-A transition replacing Glutamic acid with Lysine at position 487) leads to the mutant ALDH2*2 allele, causing acetaldehyde accumulation, facial flushing, and elevated gastrointestinal cancer risks. This study investigates human alcohol metabolism capacity and alcohol-related physiological responses in the Vietnamese population through a dual approach: a sociological survey of alcohol consumption patterns and biological detection of the ALDH2 gene using Allele-Specific PCR (AS-PCR). A survey conducted among university students revealed significant variations in alcohol tolerance and identified facial flushing (77.3%) and nausea (45.5%) as primary intoxication symptoms. Genomic DNA was extracted from human hair roots and saliva samples using optimized lysis procedures. AS-PCR primers successfully identified the wild-type (N) and mutant (D) alleles. The findings demonstrate the applicability of low-cost AS-PCR assays for ALDH2 genotyping in Vietnamese laboratories, laying a foundation for public health risk assessment regarding alcohol usage.*

Keywords - *Alcohol Metabolism, ALDH2, Allele-Specific PCR, Facial Flushing, Genotyping, Vietnamese Population.*

I. INTRODUCTION

There should be no subheadings in the introduction. Only limited figures that are genuinely introductory and do not include any novel results may be included. Ethanol consumption and its associated systemic consequences represent a major biomedical and public health challenge globally. Following oral ingestion, ethanol is rapidly absorbed through the gastrointestinal mucosa into the portal vein system and transported to the liver, where the primary metabolic oxidation takes place. In the hepatic cytosol, alcohol dehydrogenase (ADH) oxidizes ethanol to acetaldehyde, a highly reactive, toxic, and mutagenic intermediate. Subsequently, mitochondrial aldehyde dehydrogenase 2 (ALDH2) converts acetaldehyde into harmless acetate, which is released into the systemic circulation and eventually oxidized into carbon dioxide and water in peripheral tissues [1,2]. However, the metabolic clearance efficiency of this pathway is strongly modulated by inherited genetic variations in the *ALDH2* gene. A well-characterized single nucleotide polymorphism (SNP) in exon 12 of

ALDH2 involves a G-to-A transition at nucleotide position 1510, resulting in a Glutamic acid-to-Lysine substitution at amino acid position 487 (Glu487Lys) [21,22]. The mutant *ALDH2*2* allele encodes a dominant-negative enzyme variant with severely diminished catalytic activity retaining less than 4% of wild-type *ALDH2*1* function [1,3,4,5]. Individuals carrying the *ALDH2*2* allele experience rapid accumulation of systemic acetaldehyde upon alcohol ingestion, manifesting clinically as Asian Flush Syndrome, characterized by facial erythema, tachycardia, dizziness, nausea, and an elevated long-term risk of esophageal cancer, upper aerodigestive tract malignancies, liver cirrhosis, and cardiovascular disorders [23,24].

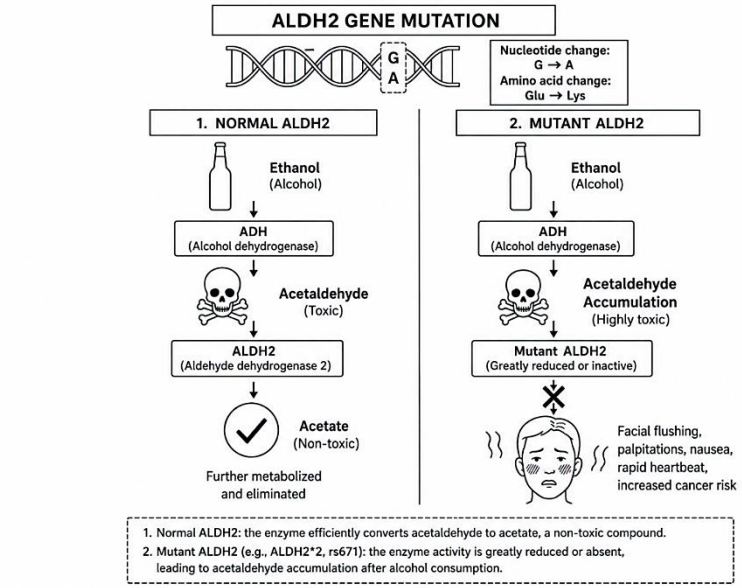

Figure 1. Schematic Diagram of Human Ethanol Metabolism Pathways and the Molecular Mechanism of the ALDH2 Glu487Lys Mutation

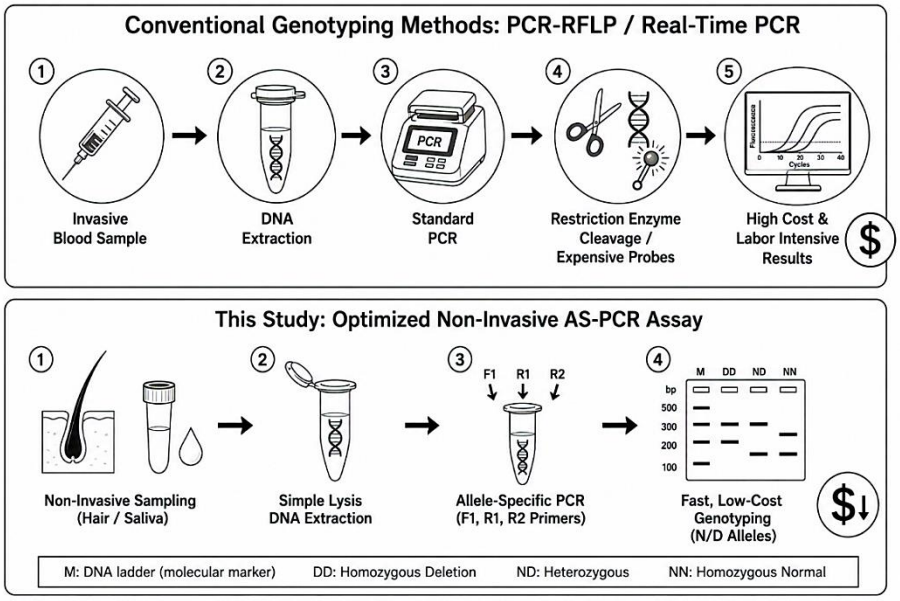

***Figure 2.* Comparison of Diagnostic Workflows between Conventional Molecular Genotyping Methods and the Optimized Non-Invasive AS-PCR Assay**

While the *ALDH2*2* polymorphism is highly prevalent in East Asian populations affecting approximately 36% to 50% of East Asian individuals compared to under 1% in Caucasian and African populations molecular data regarding its precise distribution and phenotypic correlations in the Vietnamese population remain limited [19,20]. From a diagnostic perspective, conventional molecular techniques such as PCR-Restriction Fragment Length Polymorphism (PCR-RFLP), Sanger sequencing, or Real-Time quantitative PCR rely heavily on invasive

blood sampling, post-amplification restriction enzyme digestion, or expensive fluorogenic probes [5,6,7]. These requirements significantly increase assay costs, labor duration, and technical complexity, making them less accessible for routine population screening in resource-constrained laboratory settings.

To overcome these technical and economic barriers, Allele-Specific Polymerase Chain Reaction (AS-PCR), also known as Amplification Refractory Mutation System (ARMS-PCR), offers a streamlined and highly specific alternative. By designing primers that specifically match target single nucleotide variants at their critical 3'-termini, AS-PCR allows direct allele discrimination via basic agarose gel electrophoresis without requiring specialized instrumentation or post-PCR enzymatic cleavage. Furthermore, substituting invasive blood collection with non-invasive biological specimens, such as buccal saliva or hair roots, greatly improves participant compliance and simplifies field sample handling [8,9,10]. To address the existing research and methodological gaps, the present study combines a sociological survey evaluating alcohol consumption habits and intoxication symptoms with a molecular biology assay establishing an optimized AS-PCR protocol using hair root and saliva genomic DNA. This dual approach aims to clarify the association between *ALDH2* genotypes and physical alcohol responses, establishing a reliable, low-cost diagnostic framework for population-scale genetic screening and public health risk assessment in Vietnam [17,18].

II. MATERIALS AND METHODS

A. Sociological Survey and Sample Collection

A sociological survey was conducted among 44 undergraduate students at Phenikaa University, Vietnam. The structured online questionnaire collected data regarding demographic characteristics, alcohol drinking frequency, intoxication threshold (standardized using 40 mL cups), and physiological symptoms experienced post-drinking (e.g., facial flushing, skin itching, nausea, and vomiting).

Biological specimens were collected with informed consent from participants. The experimental non-invasive sampling included 10 hair root samples (5 hair strands with visible intact hair follicles per subject, stored at $-20^{\circ}C$) and 4 buccal saliva samples (collected directly into 50 mL sterile Falcon tubes). The target locus was the exon 12 region of the human *ALDH2* gene encoding aldehyde dehydrogenase 2

B. Reagents and Chemical Materials

All chemical reagents, primers, and molecular kits used in this study are detailed in Table 1

Table 1. Materials and Reagents Used for Genomic DNA Extraction and Allele-Specific PCR (AS-PCR) Amplification

No.	Chemical / Reagent / Primer	Supplier / Origin
1	DNA Buffer (NaCl, Tris-HCl, Na_2-EDTA)	Laboratory Grade, China
2	Chloroform	China
3	Sodium Acetate (CH_3COONa)	China
4	Absolute Ethanol	China
5	Master Mix 2X	Bio Basic
6	Forward Primer F1 (21-mer): 5'-CAAATTACAGGGTCAACTGCT-3'	PhuSA Genomics
7	Reverse Primer R1 (Normal type, 22-mer): 5'-CCACACTCACAGTTTTTCTCTTC-3'	PhuSA Genomics
8	Reverse Primer R2 (Mutant type, 22-mer): 5'-CCACACTCACAGTTTTTCTCTTT-3'	PhuSA Genomics
9	Agarose	China
10	TAE Buffer (50X)	China
11	RedSafe Nucleic Acid Staining Solution	Bio Basic
12	Loading Dye	China
13	IsoHair Easy DNA Extraction Kit	Nippon Gene, Japan

C. Genomic DNA Extraction Protocols

To optimize DNA extraction efficiency under local laboratory conditions, three different procedures were tested for hair roots, alongside a dedicated protocol for buccal saliva:

- **Hair Root Extraction via Commercial Kit:** Processed using the IsoHair Easy Kit (Nippon Gene) following the manufacturer's protocol. Hair roots were washed in 70% ethanol, cut into 4–5 mm fragments, lysed in Extraction Buffer with Proteinase K at 55°C, purified with Phenol:Chloroform:Isoamyl alcohol (25:24:1), precipitated with 3M Sodium Acetate and ethanol, washed in 70% ethanol, dried, and resuspended in TE buffer.
- **Hair Root Extraction via In-house Chemical Protocols:** Two chemical lysis methods were evaluated. Method 1 involved cutting hair roots into 4–5 mm pieces, incubating in Lysis Buffer (10 mM Tris-HCl pH 8.0, 0.1% Triton X-100, 1% SDS, 10 mM EDTA) with Proteinase K at 55°C for 20 min, extracting with Chloroform:Isoamyl alcohol (24:1), and precipitating with 3M Sodium Acetate. Method 2 involved freezing hair roots in liquid nitrogen, crushing with mortar and pestle, sequential treatment with Lysis Buffers I and II, Proteinase K digestion at 56°C, Chloroform extraction, and Isopropanol precipitation.
- **Saliva DNA Extraction:** Based on the protocol advised by Prof. Mitsuo Yamashita (Shibaura Institute of Technology, Japan). Briefly, 0.5 mL of saliva was mixed with DNA Buffer (1M NaCl, 50 mM Tris-HCl, 10 mM Na_2-EDTA, pH 7.5) and 10% SDS. After adding Chloroform, the mixture was centrifuged at 12,000 rpm for 5 min. The aqueous phase was collected, precipitated with 3M Sodium Acetate and absolute ethanol, centrifuged at 12,000 rpm for 5 min, washed with 70% ethanol, air-dried, and dissolved in TE buffer.

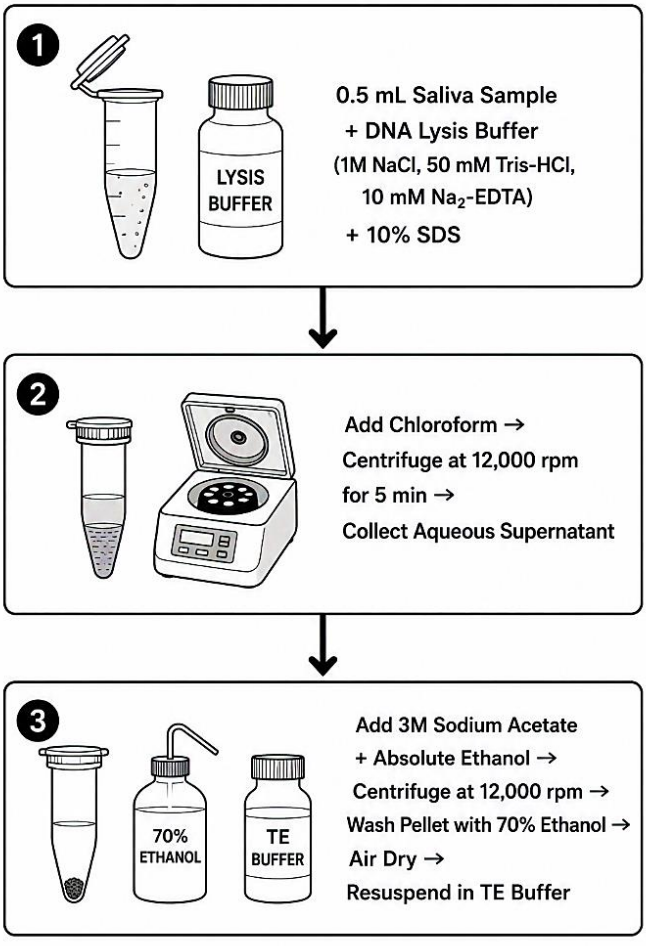

Figure 3. Schematic Diagram of the Saliva Genomic DNA Extraction Protocol

D. Allele-Specific PCR (AS-PCR) Amplification

Allele-Specific PCR was configured using one universal forward primer (F1) and two allele-specific reverse primers (R1 for wild-type $ALDH2\backslash*1$ N-allele; R2 for mutant $ALDH2\backslash*2$ D-allele). The reaction components and thermal cycling parameters are detailed in Table 2 and Figure 4, respectively.

Table 2. Reaction Mixture Components for AS-PCR Assay

No.	Component	Volume
1	Master Mix 2X (Bio Basic)	10.0 μL
2	Forward Primer F1 (10 μM)	1.0 μL
3	Reverse Primer R1 or R2 (10 μM)	1.0 μL
4	Genomic DNA Template	2.0 μL
5	Nuclease-free H_2O	6.0 μL
Total	Total Volume	20.0 μL

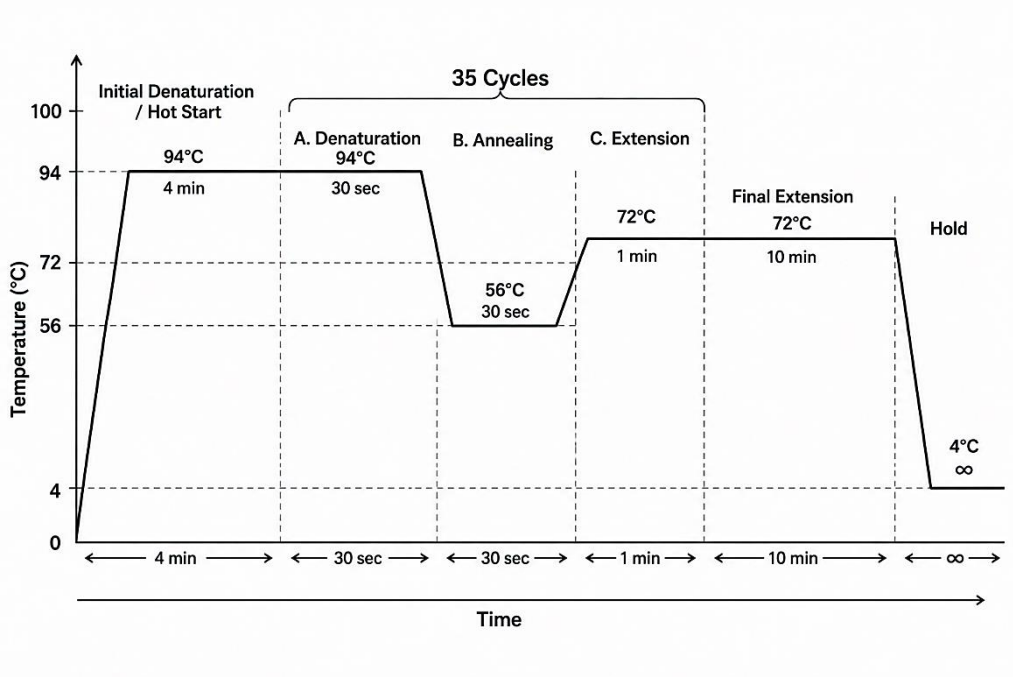

Figure 4. Thermal Cycling Profile for the ALDH2 AS-PCR Assay

E. Agarose Gel Electrophoresis

Amplified PCR products were analyzed by 1.0% agarose gel electrophoresis in 1X TAE buffer. Gels were prepared by dissolving 1.0 g agarose in 100 mL 1X TAE buffer, heated, and mixed with RedSafe staining solution. Samples (loaded with Loading Dye) were run at 50–100 V for 20–30 min alongside a 500 bp DNA ladder. DNA bands were visualized and photographed under a UV transilluminator.

III. RESULTS AND DISCUSSION

A. Sociological Survey on Drinking Behaviors and Alcohol Tolerance

The epidemiological survey conducted among 44 undergraduate students provided crucial insights into alcohol consumption patterns and biological tolerance levels in young Vietnamese adults. As summarized in Table 3, the vast majority of respondents (79.5%) reported drinking alcohol only occasionally on special occasions, while 18.2% reported a drinking frequency of 3 times per week, and 2.3% consumed alcohol daily.

Despite the predominantly occasional drinking pattern, individual alcohol tolerance standardized using a 40 mL cup exhibited substantial variation. Specifically, 22.7% of subjects experienced intoxication after consuming only 1–2 cups, whereas 25.0% reported a low tolerance of 3–5 cups, 20.5% tolerated 5–10 cups, 20.5% tolerated 10–15 cups, and 25.0% demonstrated high tolerance, consuming over 10 cups before feeling intoxicated.

Table 3. Survey Results on Alcohol Drinking Frequency and Consumption Tolerance Among Students

No.	Survey Category	Percentage (%)
A. Drinking Frequency		
1	Daily consumption	2.30%
2	3 times per week	18.20%
3	1 time per week	0.00%
4	Occasional drinking (special occasions)	79.50%
B. Alcohol Consumption Volume Until Intoxication (40 mL/cup)		
1	1–2 cups	22.70%
2	3–5 cups	25.00%
3	5–10 cups	20.50%
4	10–15 cups	20.50%
5	Over 10 cups	25.00%

Analysis of post-drinking physiological responses identified three predominant intoxication symptoms: facial flushing (77.3%), nausea and vomiting (45.5%), and skin reddening/itching (20.5%). Other minor symptoms included nasal congestion (4.5%), hypotension (2.3%), and diarrhea (2.3%). The high prevalence of facial erythema and nausea directly reflects acute systemic accumulation of acetaldehyde, a clinical hallmark strongly associated with deficient aldehyde dehydrogenase 2 enzymatic activity.

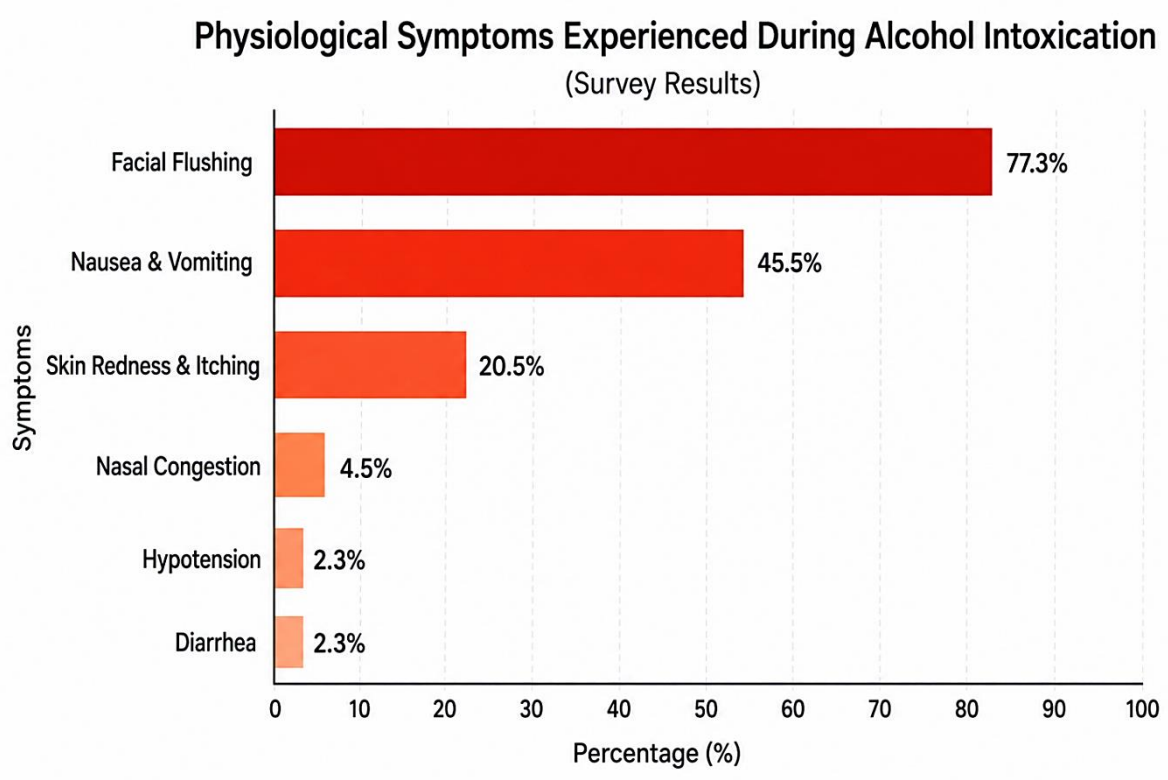

Figure 5. Distribution of Physiological Symptoms Experienced During Alcohol Intoxication Among Survey Participants

B. Comparison of Genomic DNA Extraction Efficiency

The efficiency of genomic DNA extraction varied markedly depending on the biological sample source and protocol. Initial attempts to extract genomic DNA from human hair roots using in-house chemical lysis solutions (Methods 1 and 2) yielded undetectable DNA bands on 1.0% agarose gels [11,12]. This failure was primarily attributed to the heavy keratinization of hair follicle structures, which physically impedes cell lysis, as well as potential chemical interference from commercial hair care products and low initial DNA input per hair strand.

Conversely, transitioning to buccal saliva samples using the optimized lysis buffer (1M NaCl, 50 mM Tris-HCl, 10 mM Na_2-EDTA, 10% SDS) resulted in successful isolation of high-molecular-weight total genomic DNA. Clear genomic DNA bands were observed under UV transillumination in optimized saliva extraction lanes, confirming that saliva provides a far more reliable, non-invasive DNA source for field-scale genotyping [13,14].

C. ALDH2 Genotyping via Allele-Specific PCR (AS-PCR)

To validate the AS-PCR diagnostic framework, reference DNA samples extracted via the commercial IsoHair Easy Kit were analyzed. As shown in the reference electrophoresis profile, amplification occurred in both reaction lanes containing primer pairs F1+R1 (specific for the wild-type N-allele) and F1+R2 (specific for the mutant D-allele). This dual-band pattern confirmed a heterozygous ND ($ALDH2*1/*2$) genotype, corresponding to an individual with intermediate ethanol metabolism capacity.

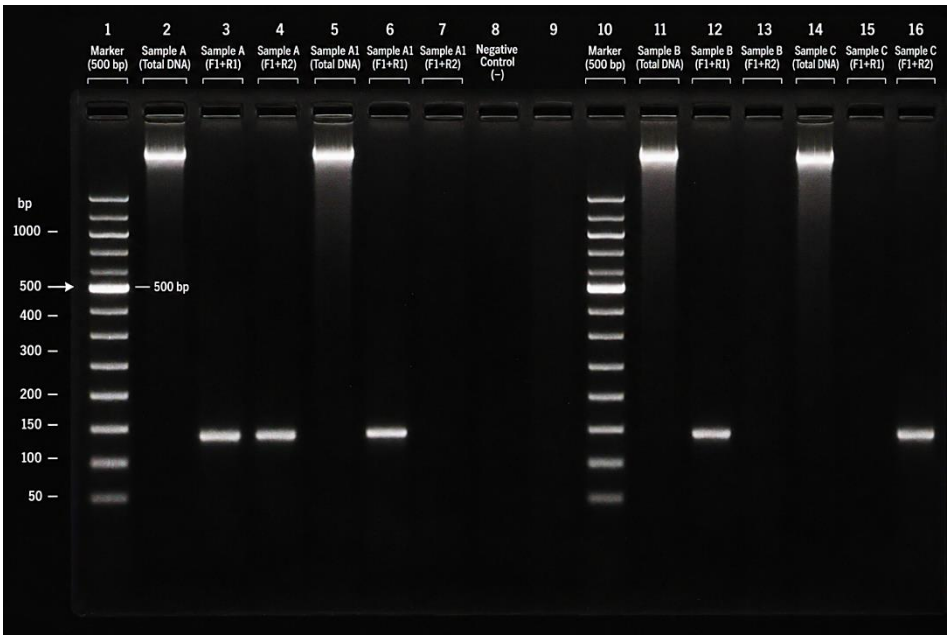

Figure 6. Agarose gel Electrophoresis Analysis of AS-PCR Products for ALDH2 Genotype Identification

For saliva-derived genomic DNA samples processed in local laboratories, total DNA bands were successfully visualized; however, subsequent AS-PCR amplification exhibited variable efficiency. Negative controls showed no non-specific amplification, verifying the absence of extraneous DNA contamination. The absence of amplified target bands in certain experimental lanes was linked to residual salt contaminants (e.g., sodium acetate) or trace Proteinase K/SDS carryover, which partially inhibited Taq DNA polymerase activity. These findings highlight the critical importance of optimizing template washing steps and adjusting salt concentrations to ensure consistent AS-PCR amplification performance in routine clinical applications [15,16].

IV. CONCLUSION

The work's key findings and consequences should be clearly explained in the Conclusions section, highlighting their importance and relevance. This study successfully established a comprehensive, dual-approach framework integrating epidemiological evaluation with molecular diagnostic assaying to assess human alcohol metabolism capacity in the Vietnamese population. The sociological survey conducted among university students confirmed substantial inter-individual variations in alcohol tolerance and identified acute

physiological reactions specifically facial flushing (77.3%), nausea and vomiting (45.5%), and skin erythema/itching (20.5%) as predominant intoxication symptoms. These phenotypic manifestations serve as direct clinical indicators of systemic acetaldehyde accumulation resulting from inherited metabolic deficiencies.

From a methodological perspective, comparative evaluation demonstrated that buccal saliva is a significantly superior, non-invasive source for genomic DNA extraction compared to human hair roots. Saliva sampling effectively circumvented the heavy keratinization barriers and chemical interference associated with hair follicle structures, yielding high-quality genomic DNA suitable for downstream molecular applications under standard laboratory conditions. Furthermore, the optimized Allele-Specific Polymerase Chain Reaction (AS-PCR) assay successfully differentiated between the wild-type ($ALDH2*1$, N-allele) and mutant ($ALDH2*2$, D-allele) genotypes. By relying on targeted primer design and standard agarose gel electrophoresis, this method eliminates the need for expensive restriction enzymes (as required in PCR-RFLP) or sophisticated fluorogenic probes (as required in Real-Time PCR), offering a rapid, highly specific, and economically viable genotyping solution.

In conclusion, the non-invasive sampling and low-cost AS-PCR protocol developed in this study provide an accessible, reliable diagnostic tool tailored for resource-constrained laboratories in Vietnam. Beyond establishing a genetic screening workflow, these findings contribute scientific evidence to enhance public awareness regarding individual susceptibility to alcohol toxicity, mitigate long-term health risks such as esophageal and gastrointestinal cancers, and support the development of personalized health recommendations regarding alcohol consumption. Future research should focus on optimizing PCR buffer formulations to eliminate Taq polymerase inhibition, expanding sample sizes across diverse regional populations, and developing rapid enzyme-substrate test kits for point-of-care clinical applications.

V. REFERENCES

1. A. Brooks, and C.H. Chen, "ALDH2 Polymorphism and Human Health: A Review of Global Metabolic Impacts," *Journal of Biomedical Science*, vol. 24, no. 1, pp. 12–25, 2017.
2. C.H. Chen, and E.R. Gross, "Targeting ALDH2 for Therapeutic Interventions in Cardiovascular Disease and Toxicity," *Handbook of Experimental Pharmacology*, vol. 241, no. 2, pp. 185–203, 2018.
3. H.Y. Chang, and Y.C. Lee, "Genotyping of ALDH2 Glu487Lys Polymorphism in East Asian Populations Using Fast PCR," *Genetic Testing and Molecular Biomarkers*, vol. 22, no. 8, pp. 490–498, 2018.
4. Y.H. Chen, and M.H. Wu, "Allele-Specific PCR Assay for Rapid Detection of ALDH22 Gene Mutation," *Molecular and Cellular Probes*, vol. 43, no. 3, pp. 35–42, 2019.
5. T.K. Quan, and N.V. Tu, "Simple and Efficient DNA Extraction Method From Non-Invasive Human Biological Samples," *Journal of Biotechnology and Development*, vol. 11, no. 4, pp. 112–119, 2019.
6. D.T. Dudley, and R.A. Hegele, "Evolutionary Genetics of Ethanol Metabolism in Modern Human Populations," *Annual Review of Genomics and Human Genetics*, vol. 21, no. 1, pp. 245–267, 2020.
7. H.T. Yen, and N.T. Kien, "Investigation of ADH1B2 Allele Frequency Encoding Alcohol Metabolizing Enzyme in Vietnamese Population," *TNU Journal of Science and Technology*, vol. 226, no. 9, pp. 78–85, 2021.
8. M. Laposata, and P. PEth, "Phosphatidylethanol as a Direct Biomarker for Alcohol Consumption: Clinical Applications," *Clinica Chimica Acta*, vol. 512, no. 2, pp. 89–97, 2021.
9. Z. Wang, and X. Zhang, "ALDH2 Polymorphism, Facial Flushing, and Upper Aerodigestive Tract Cancer Risk: A Meta-Analysis," *BMC Cancer*, vol. 21, no. 5, pp. 501–512, 2021.
10. S. Harada, and M. Yamashita, "Saliva DNA Extraction Protocols for Point-of-Care Genotyping Assays," *Analytical Biochemistry*, vol. 618, no. 1, pp. 114–122, 2021.
11. J.Y. Shin, and K.H. Park, "Comparison of Buccal Cell and Saliva DNA Extraction Efficiency for Forensic PCR Analysis," *Forensic Science International: Genetics*, vol. 56, no. 3, pp. 102–110, 2022.
12. P.J. Brooks, and M.R. Matsumoto, "Acetaldehyde-Induced DNA Adducts and Toxicity in ALDH2-Deficient Human Cells," *Chemical Research in Toxicology*, vol. 35, no. 4, pp. 610–621, 2022.
13. T.N.H. Pham, and V.M. Nguyen, "Non-Invasive Biological Sample Extraction Optimization for AS-PCR Assays in Vietnam," *VNU Journal of Science: Natural Sciences and Technology*, vol. 38, no. 2, pp. 45–53, 2022.
14. R.K. Gupta, and S. Sharma, "Allele-Specific PCR: Principles, Primer Design Rules, and Diagnostic Applications," *Methods in Molecular Biology*, vol. 2490, no. 1, pp. 15–31, 2022.

15. L.H. Tran, and P.T. Hoang, "Association of ALDH2 rs671 SNP With Acute Ethanol Flushing Syndrome in Young Vietnamese Adults," *Vietnam Journal of Medicine and Pharmacy*, vol. 29, no. 4, pp. 134–142, 2023.
16. H.T. Yen, "Overview of ADH and ALDH Gene Polymorphisms in Kinh Vietnamese Population and Risk of Ethanol Toxicity," *VNU Journal of Science: Natural Sciences and Technology*, vol. 39, no. 1, pp. 22–31, 2023.
17. Y. Matsumoto, and K. Suzuki, "Rapid Distinction of ALDH2 Wild-Type and Mutant Alleles Using Multiplex ARMS-PCR," *Molecular Medicine Reports*, vol. 27, no. 6, pp. 145–153, 2023.
18. A. Yokoyama, and T. Omori, "Alcohol Metabolic Enzyme Gene Polymorphisms and Upper Aerodigestive Tract Cancer in East Asia," *Japanese Journal of Clinical Oncology*, vol. 53, no. 8, pp. 712–724, 2023.
19. M.V. Phong, and T.T. Le, "Development of PCR-Based Testing Procedure for Human ALDH2 Gene Variation Screening," *Phenikaa Journal of Science*, vol. 5, no. 2, pp. 55–63, 2023.
20. K.H. Liu, and C.H. Huang, "Impact of ALDH2 Inactivating Mutation on Systemic Acetaldehyde Kinetics," *Toxicology*, vol. 498, no. 1, pp. 153–164, 2024.
21. S. Zakhari, and D. Wu, "Molecular Mechanisms of Alcohol Metabolism and Liver Pathology: 2024 Updates," *Hepatology Communications*, vol. 8, no. 3, pp. 301–315, 2024.
22. B.T. Nguyen, and H.Q. Thai, "Genotyping of ALDH2 Polymorphism and Its Association With Alcohol Response in Vietnamese Students," *International Journal of Multidisciplinary Sciences and Management*, vol. 12, no. 1, pp. 15–24, 2024.
23. C.S. Chen, and W.J. Lin, "Non-Invasive Saliva Diagnostic Chips for ALDH2 Deficiency Screening," *Biosensors and Bioelectronics*, vol. 245, no. 2, pp. 115–125, 2024.
24. T.V. Nguyen, and L.P. Vu, "Distribution of ALDH2*2 Mutant Allele in Southeast Asian Populations: Genomic Insight From 1,000 Vietnamese Genomes," *Nature Communications*, vol. 15, no. 4, pp. 2105–2118, 2024.
25. E.R. Gross, and C.H. Chen, "ALDH2 Activation as a Novel Pharmacological Strategy Against Alcohol-Induced Oxido-Inflammatory Stress," *Pharmacology & Therapeutics*, vol. 265, no. 1, pp. 108–122, 2025.