Association of alcohol dehydrogenase and aldehyde dehydrogenase Polymorphism with Spontaneous Deep Intracerebral Haemorrhage in the Taiwan population

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Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) encode essential alcohol-metabolizing enzymes. While alcohol use is associated with spontaneously deep intracerebral haemorrhage (SDICH), particularly in males, the activities and genetic variants of ADH and ALDH may affect SDICH development. This case-control study was conducted to identify the interaction of alcohol use and SDICH with five single-nucleotide polymorphisms (SNPs): ADH1B rs1229984, ADH1C rs2241894, ALDH2 rs671, ALDH2 rs886205, and ALDH2 rs4648328. We enrolled 208 patients with SDICH and 244 healthy controls in a Taiwanese population. ALDH2 rs671 was significantly associated with SDICH in the dominant (P < 0.001) and additive models (P = 0.007). ALDH2 rs4648328 was borderline significantly associated with SDICH in the recessive (P = 0.024) or additive models (P = 0.030). In alcohol-using patients, the ALDH2 rs671 GG genotype was associated with SDICH risk compared to the GA + AA genotype (P = 0.010). ADH1B rs1229984, ADH1C rs2241894, and ALDH2 rs886205 did not demonstrate association with SDICH. Thus, the ALDH2 rs671 GG genotype is a risk factor for SDICH. Because the genetic distributions of ALDH2 rs671 exhibited strong ethnic heterogeneity, further studies in different populations are needed to validate these findings.

Primary intracerebral haemorrhage (ICH), accounting for 22–35% of all cases of stroke in Asian populations1, is the most devastating stroke subtype with high rates of death and long-term disability in adults2,3. Asian populations have higher incidences of primary ICH than Caucasians4. Sixty to eighty percent of primary ICH cases occur at the non-lobar region, including the basal ganglia, thalamus, brain stem, and cerebellum, and are also known as spontaneously deep intracerebral haemorrhage (SDICH)5. Numerous factors, such as hypertension and alcohol use, have been proposed to contribute to SDICH development6,7.

Alcohol use was associated with an increased ICH risk7. Alcohol is primarily metabolized by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH)8. The metabolism of alcohol produces acetaldehyde, acetate, and reactive oxygen species (ROS). Excessively produced acetaldehyde and ROS, which are highly reactive and toxic by-products, are distributed throughout cell membranes and interact with certain proteins, affecting cell function and leading to organs damage. The accumulation of acetaldehyde causes oxidative damage, excessive autophagy, decreased myofilament calcium sensitivity, and impaired endoplasmic reticulum calcium-ATPase function9. Acetate metabolism involved in lipid biosynthesis in the mitochondria of brain tissues10. In animal models, toxic aldehydes enlarged the cerebral ischaemia-induced infarct area and increased oxidative stress11,12. Decreased enzymatic activity of ALDH, a condition which impairs the degradation of acetaldehyde, could be
associated with higher alcohol intoxication among East Asians compared to Caucasians\(^ {13}\). Previous studies suggested an association between genetic variants in the alcohol metabolism pathway and vascular diseases\(^ {14}\). Individuals with the \(\text{ALDH2}\) rs671 A allele have higher prevalence of hypertension, cardiovascular risk factors, and cerebral infarction\(^ {15}\). Polymorphisms in \(\text{ALDH2}\) rs671 are associated with coronary artery disease (CAD) in Chinese patients with hypertension\(^ {16}\). In the male Japanese population, the \(\text{ALDH2}\) rs671 GG genotype is associated with cerebral lacunar infarcts\(^ {17}\). In contrast, presence of the \(\text{ALDH2}\) rs671 A allele could be a risk factor for cerebral infarction in Han-Chinese population\(^ {15}\). A reduction in ALDH2 activity may interfere with endothelium angiogenesis and is associated with cerebral amyloid angiopathy\(^ {18}\).

However, the association between the genetic variants involved in alcohol metabolism and SDICH remains unclear. Here, we conducted a case-control study to investigate the associations of genetic variants in \(\text{ADH}\) and \(\text{ALDH}\), including \(\text{ADH1B}\) rs1229984, \(\text{ADH1C}\) rs2241894, \(\text{ALDH2}\) rs671, \(\text{ALDH2}\) rs886205, and \(\text{ALDH2}\) rs4648328, and SDICH in a Taiwanese population.

**Results**

**Patient characteristics.** Among the 208 cases with SDICH and 244 controls, the percentage of men (67.8\%) and those with hypertension (89.9\%) were significantly higher in the SDICH group compared to control group (men: 52.5\%, \(P = 0.001\); hypertension: 42.4\%, \(P < 0.001\), Table 1). More patients with SDICH had were exposed to alcohol (32.2\%) or smoking (44.2\%) compared to controls (alcohol use: 14.8\%, \(P < 0.001\); smoking: 19.3\%, \(P < 0.001\)). The levels of total cholesterol (184.6 ± 38.9 mg/dL) in patients with SDICH were lower compared to in controls (total cholesterol: 200.2 ± 42.9 mg/dL, \(P = 0.001\)). Alcohol use (SDICH vs controls: 46.1\% vs 26.0\%, \(P = 0.001\)) and smoking (SDICH vs controls: 63.8\% vs 35.4\%, \(P < 0.001\)) were more frequently observed in the male patients with SDICH (Table 1).

**Genotype frequency and association analysis of controls and patients with SDICH.** All single-nucleotide polymorphisms (SNPs) were in Hardy-Weinberg equilibrium in the case and control groups according to the standard \(\chi^2\) test at a significance level of 0.05. The genotype frequencies of the analysed SNPs in the case and control groups are shown in Table 2. \(\text{ALDH2}\) rs671 was significantly associated with SDICH in the dominant model (OR = 0.5, 95\% CI: 0.4–0.8, \(P < 0.001\)) and additive model (OR = 0.7, 95\% CI: 0.5–0.9, \(P = 0.007\)). The significance remained after adjusting for sex and age in the dominant model (OR = 0.6, 95\% CI: 0.4–0.8, \(P = 0.003\)) and borderline in the additive model (OR = 1.5, 95\% CI: 1.1–2.0, \(P = 0.015\)). However, these associations did not remain after further adjusting for hypertension and alcohol use. \(\text{ALDH2}\) rs4648328 could be associated with SDICH in the recessive model (OR = 2.4, 95\% CI: 1.1–5.1, \(P = 0.024\)) and additive model (OR = 1.4, 95\% CI: 1.0–1.9, \(P = 0.030\)) with boardline significance. These associations were not detected after Bonferroni correction and multivariate adjustment. The genotypic frequencies of other genetic variants were similar between the SDICH and controls.

The minor allele frequencies (MAFs) of the analysed SNPs in the case and control groups are shown in Table 3. The MAF of \(\text{ALDH2}\) rs671 (21.9\%) in the SDICH group was significantly lower compared to controls (30.1\%, odds ratio (OR) = 0.7, 95\% confidence interval (CI): 0.5–0.9, \(P = 0.005\)). The MAFs of the other SNPs were similar between the SDICH and control groups.

We further stratified the allelic and genotypic frequencies of \(\text{ADH1B}\) rs1229984, \(\text{ADH1C}\) rs2241894, \(\text{ALDH2}\) rs671, \(\text{ALDH2}\) rs886205, and \(\text{ALDH2}\) rs4648328 according to alcohol use. When stratified by alcohol use, the \(\text{ALDH2}\) rs671 GA genotype was significantly associated with SDICH in the alcohol use group (OR = 0.2, 95\% CI: 0.1–0.7, \(P = 0.008\)), indicating the interaction between the \(\text{ALDH2}\) rs671 genotype and alcohol use. Specifically, in alcohol-free subjects, the SDICH risk was similar between genotypes. In subjects with alcohol use, SDICH was more frequently observed in individuals carrying \(\text{ALDH2}\) rs671 GG genotype compared to rs671 GA+AA genotype (SDICH percentage: GG vs GA+AA: 70.6\% vs 38.9\%, OR = 0.3, 95\% CI 0.1–0.8, crude \(P = 0.01\), Fig. 1), whereas this difference was not observed after multivariable adjustment. There was no association between all tested SNPs and SDICH by stratification according to the presences of hypertension and gender (data not shown). None of the alleles and genotypes in this study showed associations with hypertension (Supplementary Table S1).

We further characterized the \(\text{ALDH2}\) SNPs by linkage disequilibrium (LD) and haplotype analyses. LD analysis showed that rs4648328 and rs671 were highly correlated with each other (Fig. 2). Haplotype analyses...
Analysis demonstrated that the haplotype “GT” of rs671-rs4648328 was associated with SDICH (OR = 1.4; 95% CI: 1.0–1.8, P = 0.047, Table 4). In contrast, haplotype “AC” demonstrated protective effect on SDICH (OR = 0.6; 95% CI: 0.5–0.9, P = 0.005).

Discussion
This study, at the first time, describes the potential association between SNPs of ADH and ALDH2 with SDICH susceptibility in the Taiwanese population. Asian populations have higher incidences of SDICH with high mortality and long-term disability than Caucasians.2,3,19. Alcohol use demonstrates the association with SDICH.7. Alcohol is primarily metabolized by ADH and ALDH.8. Our results support a role for \textit{ALDH2} genetic variants in SDICH. We found that the \textit{ALDH2} rs671 GG genotype could be a risk locus for SDICH, particularly in subjects who used alcohol, in Taiwanese population. Haplotype analysis further identified the association between haplotypes in rs671-rs4648328 of \textit{ALDH2} and SDICH. Further large case-control cohorts in multi-ethnicities are needed to validate this association.

The rs671 is a functional SNP (Glu504Lys) in \textit{ALDH2}.20. Minor allele (A allele) of rs671 results in reduced \textit{ALDH2} enzymatic activity. Approximately 30% of people in Asia and 47% of those in Taiwan carrying the rs671 A allele.21–24. In the male Japanese population, the \textit{ALDH2} rs671 GG genotype is associated with cerebral lacunar infarcts.17. \textit{ALDH2} rs671 A allele are associated with coronary artery disease in Chinese patients with hypertension.16. Moreover, \textit{ALDH2} rs671 A allele is also associated with hypertension and cerebral amyloid angiopathy.18,25. Although \textit{ALDH} rs671 AA genotype may be associated with alcoholism-related hypertension,26. our results did not detect the association between \textit{ALDH2} rs671 and hypertension, supporting the primary effect of \textit{ALDH2} rs671 on SDICH. \textit{ALDH2} rs671 GG genotype tends to be a risk factor for SDICH, particularly in the group with alcohol use in Taiwanese.

Table 2. Genotypes of the SNPs and their associations with risk of spontaneously deep intracerebral haemorrhage (SDICH). SDICH: spontaneous deep intracerebral haemorrhage, OR: Odds ratio, CI: confidence interval. Analysis were performed by logistic regression under dominant, additive and recessive genetic models. Model 1: Crude logistic regression. Model 2: Multivariable logistic regression, adjust sex, age. Model 3: Multivariable logistic regression, adjust sex, age, HTN and alcohol. P-value with Bonferroni correction for significance was 0.01.
ALDH2 rs4648328, an intronic SNP, was associated with delayed alcohol metabolism in European population. In our analysis, we found a potential association between rs4648328 and SDICH in the recessive and additive models. SNPs in ALDH2 demonstrated strong LD in Indian population. In addition, our study showed that rs4648328 was in LD with rs671 in Taiwanese population. The haplotype “GT” of ALDH2 rs671-rs4648328 was associated with SDICH, whereas the haplotype “AC” demonstrated protective effect on SDICH. This study provides a baseline for future research about the role of the ALDH2 loci in SDICH in Taiwanese population. Further large-scale investigations are needed to confirm this result.

Table 5 shows ethnicity differences in SNPs of ADH and ALDH. The genetic distributions of ALDH2 rs671 showed strong ethnic heterogeneity. The frequencies of A allele in Taiwanese (26.3%) and East Asians (17.4%) are much higher compared to Americans (0%), Europeans (0%) and global populations (3.6%). Previous studies showed that genetic variants of ALDH2 rs671 were associated with both alcohol flushing and alcohol use in Asian populations. Additionally, the ALDH2 rs671 GG genotype is associated with cerebral lacunar infarcts in the male Japanese. Our study showed that rs671 GG genotype was associated with SDICH susceptibility, particularly in the alcohol use group.

In addition to rs671, the MAF T allele was present in 15.9% of rs1229984 in global population, while the rs1229984 C allele was present in 26.2% of Taiwanese and 30.3% of east Asian. (Table 5). While the ADH1B rs1229984 CC genotype is predominant in East Asian population, it is rarely observed in Indian population. The role of ADH1B rs1229984 in modulating alcohol consumption remains controversial. It has been reported that ADH1B rs1229984 C allele is associated with alcoholism. However, a case-control study suggests that CC genotype of ADH1B rs1229984 may protect against alcohol dependence. In our analysis, the ADH1B rs1229984 did not demonstrate association with alcohol consumption.

| Gene | SNP ID | All cases MAF | MAF Model 1 | Control (%) | OR (95% CI), P value | P value | P value |
|------|--------|---------------|-------------|-------------|----------------------|---------|---------|
| ALDH2 | rs671  | A/0.263       | 0.219       | 0.301       | 0.7 (0.5–0.9), 0.005 | 0.012   | 0.523   |
|      | rs4648328 | T/0.249     | 0.284       | 0.219       | 1.4 (1.0–1.9), 0.026 | 0.065   | 0.543   |
|      | rs886205 | A/0.141      | 0.135       | 0.146       | 0.640                | 0.620   | 0.528   |
|      | rs1229984 | C/0.262     | 0.269       | 0.256       | 0.655                | 0.671   | 0.617   |
|      | rs2241894 | T/0.282     | 0.284       | 0.281       | 0.923                | 0.899   | 0.365   |

Figure 1. Interaction between ALDH2 rs671 genotype and alcohol use to SDICH susceptibility. Comparisons between controls and ICH group were analysed by logistic regression under alcohol use or not. Although the interactive effect between alcohol use and rs671 genotype was borderline significant (P = 0.07), in those with alcohol use, the ALDH2 rs671 GG genotype was a significant risk for SDICH compared to the rs671 GA+AA genotype (SDICH percentage: GG vs GA+AA: 70.6% vs 38.9%, OR = 0.3, 95% CI 0.1–0.8, P = 0.014) while adjusting for sex and age. In contrast, the SDICH risk was similar between genotypes (P = 0.21) in alcohol-free subjects. *Crude logistic regression. #Multivariable logistic regression, adjust for sex and age.
The MAF C allele was present in 47.2% of rs2241894 in global population, while the rs2241894 T allele was present in 28.2% of Taiwanese. A genome-wide association study also demonstrated an association between ADH1C rs2241894 and alcohol dependence in African and European Americans.14 The MAF A allele was present in 49.1% of rs886205 in global population, while the rs886205 A allele was present in 14.1% of Taiwanese. A recent study reported that ALDH2 rs886205 is associated with alcohol-dependent patients.33 However, we found no associations between ALDH2 rs886205, ADH1C rs2241894, alcohol use and SDICH in our analysis. This discrepancy may be contributed by the ethnic difference of genetic background, as well as the design of studies.

To our knowledge, this is the first study to propose that the ALDH2 rs671 GG genotype is a risk factor for SDICH, particularly in an alcohol-using population. ALDH2 rs671 and rs4648328 are particularly important in the interaction with alcohol use, one of the major environmental risk factors for SDICH. There are limitations to our study. First, this was a hospital-based study which may limit the generalization of our results to the whole population. Most

![Figure 2.](image-url)

**Figure 2.** Linkage disequilibrium (LD) between the SNP markers in ALDH2 in the Taiwanese population. Graphical representation of SNPs in Haploview linkage disequilibrium (LD) of ALDH2 gene in SDICH patients and controls. Haploview LD coefficients D' × 100 were generated by Haploview 4.2 and shown in each cell using the standard color scheme. D' values of “0” indicates the independence of the examined two loci while a value of “1” demonstrates complete linkage. The strength of LD is depicted by red intensity, which moves from white to red as D' × 100 progresses from 1 to 100. Two SNPs (rs671 and rs4648328) constitute one haplotype block that span 18kb of ALDH2 gene with strong linkage disequilibrium (LD), shown in bright red (D': 0.97; r²: 0.11). The LD values were presented as D': 0.99 (r²: 0.05) between rs671 & rs886205 and D': 0.63 (r²: 0.02) between rs4648328 & rs886205 respectively.

| Genotypes | Case (freq%) | Control(freq%) | OR (95% CI) | Fischer's P |
|------------|--------------|----------------|-------------|-------------|
| rs671      | rs4648328    |                |             |             |
| Haplotype  |              |                |             |             |
| Hap1 A C   | 21.5         | 29.8           | 0.6 (0.5 – 0.9) | 0.005       |
| Hap2 G T   | 28.0         | 22.4           | 1.4 (1.0 – 1.8) | 0.047       |
| Hap3 G C   | 50.1         | 47.8           | 1.1 (0.9 – 1.4) | 0.452       |

**Table 4.** The association between haplotypes of ALDH2 genetic polymorphisms and the risk of spontaneously deep intracerebral hemorrhage (SDICH). ALDH, aldehyde dehydrogenase; CI, confidence interval; Hap, haplotype; OR, odds ratio.
Table 5. Minor allele frequency (MAF) in different populations. SNP: Single-nucleotide polymorphism; MAF: minor allele frequency. *MAF data from 1000 genome information.

| Gene | SNP ID | Sample size | Present study | Globala | East Asiana | South Asiana | Americana | Europea | Africaa |
|------|--------|-------------|---------------|---------|------------|-------------|-----------|---------|---------|
|      |        | N = 452    | N = 5008      | N = 1008 | N = 987    | N = 694     | N = 1006  | N = 1322 |
| ALDH2| rs671  | A/0.263    | A/0.206       | A/0.174 | A/0.000    | A/0.000     | A/0.000   | A/0.002  |
|      | rs4648328 | T/0.249     | T/0.200       | T/0.263 | T/0.210    | T/0.150     | T/0.159   | T/0.204  |
|      | rs886205 | A/0.141    | A/0.491       | A/0.156 | G/0.290    | G/0.310     | G/0.166   | A/0.223  |
| ADH  | rs1229984 | C/0.262     | T/0.159       | C/0.303 | T/0.020    | T/0.060     | T/0.029   | T/0.002  |
|      | rs2241894 | T/0.282     | C/0.472       | T/0.236 | T/0.400    | C/0.170     | C/0.231   | C/0.495  |

Conclusion. This study revealed a significant association between the genetic variants of ALDH2 and SDICH susceptibility. Carrying the ALDH2 rs671 GG genotype tends to be a risk factor for SDICH, particularly in those who use alcohol.

Materials and Methods

Patients and control subjects. Patients (age ≥ 30 years old) with SDICH at the basal ganglia, thalamus, cerebellum, or brainstem were included in the study. The size and location of SDICH were confirmed by brain computed tomography (CT). Patients with traumatic cerebral haemorrhage, haemorrhagic transformation of a cerebral infarct, vascular anomaly, and secondary intracranial haemorrhage (coagulopathy or hyper-perfusion syndrome) were excluded. Controls were defined as those without medical disease such as renal failure, myocardial infarction, cancer, stroke history, and neurodegenerative disease. A history of hypertension, diabetes mellitus, smoking, alcohol use, and lipid profile were collected from all participants. Alcohol use referred to the consumption of greater than 210 g of alcohol per week. Smokers were defined as former or current smokers.

This retrospective case-control study was approved by the Chang Gung Memorial Hospital Institutional Ethics Review Board for human studies, and patients provided written informed consent prior to study participation (IRB201600775B0). All methods were performed in accordance with the relevant guidelines and regulations.

Selection of SNP and genotyping. The cytogenetic location of ALDH2 is 12q24. In the literature review, approximately 30% people in Asia and 47% in Taiwan were described to carry genetic variants of the A allele in ALDH2 with reduced enzymatic activity. We selected the ALDH2 rs671 (G > A, missense variant Glu504Lys, exon 12), ADH1B rs4648328 (C > T, intron variant, intron 3), and ADH2 rs886205 (G > A, promoter, 5′-untranslated region) based on previous evidence of its association with alcohol dependence. For ADH1 (cytogenetic location at 4q22), we selected ADH1B rs1229984 (T > C, missense variant Arg48His, exon 3) and ADH1C rs2241894 (A > G, synonymous variant Thr151, exon 5). ADH1B rs1229984 was previously investigated for its association with alcohol metabolism and alcohol drinking behaviours. Additionally, ADH1B rs1229984, ADH1C rs2241894, and ALDH2 rs671 are greatly different between Asians and Caucasians.

Blood samples were collected for genotyping. The genomic DNA was extracted from peripheral leukocytes by using the Stratagene DNA extraction kit (La Jolla, CA, USA). Polymorphisms were genotyped using TaqMan SNP Assays in the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The primer sets used for polymerase chain reaction amplification of each SNP region are as listed in Supplementary Table S2. Each SNP was checked for Hardy-Weinberg equilibrium using the standard χ² test at a significance level of 0.05. Patterns of LD and haplotype analyses were evaluated using SHEsis Online Version (http://analysis.bio-x.cn/myAnalysis.php). Haplotypes with frequency <3% were excluded from association analysis.

Statistical analysis and power estimation. All data analyses were performed using SAS Software (version 9.4; SAS Institute, Cary, NC, USA). Demographic data and the distributions of genotypes of SNPs were analysed by χ² test, t-test, and univariate logistic regression. Multivariable logistic regression analyses were used to test the null hypothesis that the number of cases and controls did not differ with various genotypes of the five SNPs. Potential covariates included age, sex, hypertension, total cholesterol level, and alcohol use. Samples were stratified by alcohol use using multivariable logistic regression. All P values were two-tailed. While considering Bonferroni correction, the significance level was set to 0.01. Given the observed allele frequency in the present case-control study, at the 0.01 significance level, we had power greater than 0.8 to identify an association of the genetic variant with SDICH susceptibility when the per-allele genetic effect was greater than an odds ratio of 1.8 for rs886205 and 1.7 for the rest of the SNPs.
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Author contributions
Conceived the study: Y.C.C. Designed the experiments: Y.C.C. and Y.S.L. Performed the laboratory work: Y.C.C., Y.S.L., K.H.C. and C.M.C. Analyzed and interpretation of data: Y.H.H. and Y.S.L. Wrote the first draft of manuscript: Y.H.H. and K.H.C. Wrote and revised the final version of manuscript: Y.H.H. and Y.C.C.

Competing interests
The authors declare no competing interests.

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