Association between ALDH2 polymorphism and esophageal cancer risk in South Koreans: a case-control study

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Abstract

Background: Alcohol consumption is a major risk factor for esophageal cancer; however, a high incidence of esophageal cancer is observed particularly among Eastern Asians, although they consume relatively less alcohol, presumably due to the high frequency of aldehyde dehydrogenase 2 (ALDH2) rs671 polymorphisms. Nevertheless, the association between ALDH2 polymorphisms and esophageal cancer remains under debate. In the present study, we evaluated the association between ALDH2 rs671 polymorphisms and the risk of esophageal cancer in the South Korean population.

Methods: This study included 783 hospital based-cases and 8732 population-based controls. Information on smoking history and alcohol consumption was obtained from the medical records or interview questionnaires. Age-adjusted logistic regression analysis was performed to assess the association between ALDH2 rs671 polymorphisms and esophageal cancer.

Results: Odds ratios (ORs) for esophageal cancer in men with GA and AA genotypes were 2.75 (95% confidence interval [CI]: 2.34–3.23) and 0.08 (95% CI: 0.00–0.35), respectively; whereas, in women, these ratios were 2.99 (95% CI: 1.43–6.34) and 6.18 (95% CI: 1.40–19.62), respectively, taking subjects with the ALDH2 GG genotype as a reference. In men, the association between ALDH2 polymorphisms and esophageal cancer was modified by alcohol consumption.

Conclusion: In Eastern Asians, ALDH2 rs671 polymorphisms are associated with esophageal cancer, which may be linked to acetaldehyde accumulation.

Keywords: ALDH2, Alcohol drinking, Case-control studies, Esophageal cancer

Background

Alcohol consumption is a primary risk factor for esophageal cancer. Despite limited alcohol consumption [1], the incidence of esophageal cancer in Eastern Asia is high [2]. Moreover, in contrast to other populations, a characteristic association between less alcohol consumption and high risk of esophageal cancer is observed in Asians [3]. These epidemiological data suggest that Eastern Asians are more susceptible to the carcinogenic effects of alcohol.

The enzyme aldehyde dehydrogenase (ALDH) produces acetaldehyde from alcohol. Acetaldehyde is toxic, and its accumulation is the primary cause of unfavorable symptoms occurring after alcohol consumption. Several enzyme polymorphisms involved in alcohol metabolism affect the degree of acetaldehyde accumulation after
alcohol consumption, which varies among individuals of different ethnicities [4]. Aldehyde dehydrogenase 2 (ALDH2) rs671 polymorphisms are particularly common among East Asians, and affect alcohol metabolism [5–8]. Compared to individuals with functional ALDH2, inactive ALDH2 carriers present high acetaldehyde levels after alcohol consumption [9], which can lead to flushing, headache, palpitations, and other unfavorable symptoms that affect alcohol consumption habits; thus, minor allele A of ALDH2 rs671 polymorphisms is associated with low alcohol consumption and alcohol abstinence.

Although the ALDH2 rs671 GA genotype has been associated with a high risk of esophageal cancer [10–13], this association could not be replicated by certain studies [14, 15]. Furthermore, the relationship between ALDH2 polymorphisms and esophageal cancer has not been assessed in the South Korean population to date. Therefore, the present study aimed to evaluate the association between ALDH2 rs671 polymorphisms and the risk of esophageal cancer in South Koreans.

Methods
Study population
The case group was consecutively recruited, and comprised 834 patients with histologically confirmed esophageal cancer diagnosed at the Chonnam National University Hwasun Hospital between April 2004 and September 2014. Patients with secondary or recurrent tumors were excluded. Since control subjects were drawn from a community-based cohort study of adults aged 50 years and older, 51 patients under 50 years of age were excluded from the study. Esophageal cancer was classified into different histological types, such as squamous cell carcinoma and adenocarcinoma. The control group included participants from the Dong-gu study, which is a prospective cohort study of risk factors for chronic diseases including stroke, coronary heart disease, cognitive decline, cancer, and fracture [16]. The Dong-gu study comprised 9260 Korean adults aged 50 years and older. Of these, 8805 participants without a cancer history were included. Moreover, participants with missing data were also excluded from the study; hence, our analysis included data from 783 cases and 8732 controls. All patients and control subjects provided informed written consent to participate in this study at the time of peripheral blood collection.

Genotyping
Genotyping was performed as previously described [17]. In brief, genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. ALDH2 rs671 polymorphism genotyping was performed via high-resolution melting (HRM) analysis using a Rotor-Gene 6000TM (Corbett Research, Sydney, Australia).

Covariates
Information on smoking and drinking history in the case group was obtained retrospectively from medical records, and in the control group, obtained from interview questionnaires in the baseline survey of the Dong-gu Study. Patients were categorized as current drinkers or nondrinkers, whereas alcohol consumption in the control subjects was evaluated based on the amount and frequency of alcohol consumption, referring to the Dong-gu study. Accordingly, we defined current drinkers as individuals who reportedly consumed one or more drinks per month, and thus, classified the participants as current drinkers or nondrinkers. Smoking status was categorized as smokers (current or ex-smokers) or nonsmokers.

Statistical analysis
The baseline characteristics of patients and control participants were assessed based on their sex. Moreover, general characteristics between groups were compared using t-test for continuous variables and chi-squared test for categorical variables.

The relationship between ALDH2 rs671 polymorphisms and esophageal cancer was evaluated via age-adjusted logistic regression analysis. Due to the small sample size of participants with AA genotypes, ALDH2 rs671 polymorphism was classified as GG and GA/AA. Subjects with the ALDH2 GG genotype were used as a reference. P-values lower than 0.05 were considered as statistically significant. All analyses were performed using R software (version 3.6.3, Vienna, Austria).

Results
Table 1 summarizes the baseline characteristics of the study participants based on their sex. Esophageal cancer patients were younger than the control subjects for both sexes. The frequency of current drinkers was higher in men than in women. Squamous cell carcinoma was the most common histological type of esophageal cancer, followed by adenocarcinoma.

Figure 1 illustrates the drinking status based on ALDH2 rs671 genotypes and sex. The prevalence of current drinkers in men with ALDH2 GG and GA/AA genotypes was 74.6 and 41.1%, respectively; whereas in women, it differed slightly as women with the GG genotype (24.3%) revealed less prevalence than those with the GA/AA genotype (6.5%). In contrast, the prevalence of smoking was not related to the ALDH2 rs671 genotypes regardless of sex (Fig. 2).

Table 2 summarizes the distribution of ALDH2 rs671 genotypes, as well as the age-adjusted odds ratio (OR)
and 95% confidence interval (CI) for esophageal cancer. Using subjects with the \textit{ALDH2} GG genotype as a reference, ORs for esophageal cancer among male individuals with GA and AA genotypes were 2.75 (95% CI, 2.34–3.23) and 0.08 (95% CI, 0.00–0.35), respectively, whereas those in women were 2.99 (95% CI, 1.43–6.34) and 6.18 (95% CI, 1.40–19.62), respectively.

Table 3 summarizes the results of subgroup analysis of the association between \textit{ALDH2} rs671 polymorphism and esophageal cancer. In current drinkers, \textit{ALDH2} genotypes had a greater impact on esophageal cancer risk than in nondrinkers. In men, the association between \textit{ALDH2} rs671 polymorphisms and esophageal cancer differed significantly based on alcohol consumption (\(P < 0.001\)). Using subjects with the \textit{ALDH2} GG genotype as a reference, the OR of the \textit{ALDH2} GA/AA genotype among current drinkers was 4.39 (95% CI, 3.54–5.46) in men and 14.45 (95% CI, 2.45–89.48) in women; however, among the nondrinkers, it was 1.25 (95% CI, 0.97–1.61) in men and 2.95 (95% CI, 1.35–6.76) in women. Smoking history did not affect the association between \textit{ALDH2} polymorphisms and esophageal cancer in either sex.

**Discussion**

This is the first study to evaluate the association between \textit{ALDH2} rs671 and esophageal cancer in the South Korean population. Several lines of evidence support the relationship between \textit{ALDH2} rs671 polymorphism and alcohol consumption. In this study, we found that the \textit{ALDH2} GA/AA genotype is associated with a high risk of esophageal cancer. Notably, the \textit{ALDH2} genotype had a higher impact on the risk of esophageal cancer in current drinkers than that in nondrinkers.

In accordance with our findings, previous studies reported a relationship between the \textit{ALDH2} GA/AA
genotype and the risk of esophageal cancer. An exploratory genome-wide association study (GWAS) revealed that ALDH2 rs671 polymorphisms were associated with esophageal cancer [10]. The study also reported that the GA and AA genotypes were associated with high and low risk of esophageal cancer, respectively. Moreover, case-control studies [11, 13] and a prospective cohort study [12] suggested an association between ALDH2 rs671 polymorphisms and esophageal cancer. In the Kadoorie Biobank cohort [12], individuals with minor allele of ALDH2 rs671 polymorphism presented high risk of esophageal cancer, and the impact of ALDH2 polymorphisms on esophageal cancer risk was higher among individuals who consumed alcohol. Nevertheless, these results could not be replicated by other studies, which reported that minor allele of ALDH2 rs671 polymorphisms were not associated with esophageal cancer [15], or were associated with a low risk of esophageal cancer [14]. These discrepancies could be attributed to differences in average alcohol consumption [1, 5], polymorphism distribution across populations [4], or various alcohol consumption definitions. Furthermore, the relationship between ALDH2 polymorphisms and alcohol consumption differed based on sex [5], thereby leading to contradictory findings among studies that did not perform sex-stratified analyses. During subgroup analysis for nondrinkers, the ALDH2 GA/AA genotype revealed a higher risk of esophageal cancer than the ALDH2 GG genotype in women, unlike in men, presumably due to distinct hormonal receptors in both sexes. ALDH2 may be unable to efficiently detoxify the endogenous aldehydes related to carcinogenesis in the ALDH2 GA/AA genotype when compared to that in the ALDH2 GG genotype [18]. It is assumed that the estrogen receptors cause differences in the relationship between ALDH2 rs671 polymorphism and carcinogenesis [19]; however, the biological mechanism between ALDH2 and hormonal receptors needs to be further evaluated.

Moreover, differences in exposure to salivary acetaldehyde among individuals with distinct genotypes may impact the effect of the ALDH2 genotype on esophageal cancer risk. The salivary acetaldehyde levels after ethanol consumption were higher in ALDH2-deficient individuals compared to individuals with functional ALDH2;

|                       | Men                              | Women                             |
|-----------------------|----------------------------------|-----------------------------------|
|                       | Case/Control OR (95% CI)         | Case/Control OR (95% CI)          |
| GG                    | 334/2338 1 (reference)           | 13/3682 1 (reference)             |
| GA                    | 416/1048 2.75 (2.34–3.23)        | 16/1440 2.99 (1.43–6.34)          |
| AA                    | 1/91 0.08 (0.00–0.35)            | 3/133 6.18 (1.40–19.62)           |
| GA + AA               | 417/1139 2.54 (2.16–2.98)        | 19/1573 3.25 (1.61–6.76)          |

OR: Odds ratio, CI: Confidence interval
this difference was observed after consuming two to three drinks [9, 20]. Thus, alcohol consumption can increase the acetaldehyde levels in esophagus, particularly in individuals with ALDH2 GA/AA genotypes with slow acetaldehyde metabolism. Acetaldehyde associated with alcoholic beverages has been classified as a group 1 carcinogen by the International Agency for Research on Cancer [21]. Acetaldehyde reacts with DNA, generating unstable DNA adducts and inducing DNA damage [22]. In a study involving Japanese alcoholic patients, the ALDH2 GA genotype was associated with high levels of N2-ethylidene-di-deoxyguanosine, the most common DNA adduct induced by acetaldehyde [23].

The present study has several limitations. First, the number of patients with AA genotypes was small; therefore, we could not perform subgroup analysis to evaluate the risk of esophageal cancer according to the genotype. Second, we did not assess the role of alcohol dehydrogenase 1B (ADH1B) rs1229984 polymorphisms, which are common in East Asians. Third, the dose-response relationship between alcohol consumption and esophageal cancer risk could not be assessed, because the amount of alcohol consumption was not evaluated in the case group. It is necessary to assess the relationship between genetically predicted alcohol consumption and esophageal cancer through a two-stage or two-sample Mendelian randomization study.

Conclusions
ALDH2 rs671 polymorphisms are associated with esophageal cancer in Eastern Asians. Notably, individuals with ALDH2 GA/AA genotypes who consumed alcohol were at a particularly high risk of esophageal cancer, presumably due acetaldehyde accumulation in the esophagus. By answering a questionnaire on alcohol flushing response, high-risk individuals with low-activity ALDH2 can be identified noninvasively without genotyping resources [8, 12], and esophageal cancer prevention programs such as interventions for alcohol consumption cessation or screening programs can be effectively implemented [24].

Abbreviations
ALDH: Aldehyde dehydrogenase; OR: Odds ratio; CI: Confidence interval; GWAS: Genome-wide association study; ADH1B: Alcohol dehydrogenase 1B

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Authors’ contributions
MHS conceived and coordinated the study. CKC and MHS drafted manuscript. CKC, JY, and MHS statistical analysis. CKC, JY, SSK, SHC, HYK, EM, and MHS performed manuscript revision. SSK, SHC, and MHS contributed to clinical data management and were involved in the recruitment of the patients and controls. All authors approved the final manuscript.

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Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
All patients and control subjects provided informed consent to participate in this study at the time of peripheral blood collection. This study was reviewed and approved by the Institutional Review Boards (IRBs) of Chonnam National University Hospital Hwasun (BBRB-2007-3) and Chonnam National University Hospital (I-2008-05-056).

Consent for publication
Not applicable.

Table 3 Distribution of ALDH2 rs671 genotype and age-adjusted odds ratio for esophageal cancer according to sex, alcohol consumption, and smoking history

|                | Men Case/Control | OR (95% CI) | Women Case/Control | OR (95% CI) |
|----------------|------------------|-------------|--------------------|-------------|
| **Current drinker** |                  |             |                    |             |
| GG             | 211/1782         | 1 (reference) | 3/896              | 1 (reference) |
| GA + AA        | 219/421          | 4.39 (3.54–5.46) | 3/101              | 14.45 (2.45–89.48) |
| **Nondrinker** |                  |             |                    |             |
| GG             | 123/556          | 1 (reference) | 10/2786            | 1 (reference) |
| GA + AA        | 198/718          | 1.25 (0.97–1.61) | 16/1472            | 2.95 (1.35–6.76) |
| **Ever smoker** |                  |             |                    |             |
| GG             | 255/1734         | 1 (reference) | 2/155              | 1 (reference) |
| GA + AA        | 301/842          | 2.41 (2.00–2.91) | 1/35               | 2.02 (0.09–22.02) |
| **Never smoker** |                 |             |                    |             |
| GG             | 79/604           | 1 (reference) | 11/3527            | 1 (reference) |
| GA + AA        | 116/297          | 2.95 (2.15–4.07) | 18/1538            | 3.49 (1.67–7.67) |

OR Odds ratio, CI Confidence interval
Competing interests
The authors declare that they have no competing interests.

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