Genetic polymorphisms in cytochrome P4502E1, alcohol and aldehyde dehydrogenases and the risk of esophageal squamous cell carcinoma in Gansu Chinese males

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Abstract

AIM: To evaluate the association between genetic polymorphisms in CYP2E1, ADH2 and ADH1B and the risk of esophageal squamous cell carcinoma (ESCC) in a high risk area of Gansu Province, in Chinese males.

METHODS: A case-control study was conducted to investigate the genetic polymorphisms of these enzymes (CYP2E1*c1/*c2, *c1/*c2, CYP2E1, *1/*2 and ADH1B frequencies were higher among patients with squamous cell carcinomas, at a level close to statistical significance (P = 0.014; P = 0.094; P = 0.0001 respectively). There were synergistic interactions among alcohol drinking and ADH2, ADH1B and CYP2E1 genotypes. The risk of the ESCC in moderate-to-heavy drinkers with an active ALDH2 (*1/*1 genotype) as well as ADH1B (*1/*2 + *2/*2) and CYP2E1 (*c1/*c2 + *c2/*c2) genotypes, with a statistically significant difference; ORs (95% CI) of 8.58 (3.28-22.68), 27.12 (8.52-70.19) and 7.64 (2.82-11.31) respectively. The risk of the ESCC in moderate-to-heavy drinkers with ALDH2 (*1/*2) combined the ADH1B (*1/*1) genotype or ALDH2 (*1/*2) combined the CYP2E1 (*1/*c1) genotype leads to synergistic interactions, higher than drinkers with ALDH2 (*1/*1) + ADH1B (*1/*2 + *2/*2), ALDH2 (*1/*1) + CYP2E1 (*c1/*c2 + *c2/*c2) respectively , ORs (95% CI) of 7.46 (3.28-18.32) and 6.82 (1.44-9.76) respectively. Individuals with the ADH1B combined the CYP2E1 genotype showed no synergistic interaction.

CONCLUSION: In our study, we found that alcohol consumption and polymorphisms in the CYP2E1, ADH1B and ALDH2 genes are important risk factors for ESCC, and that there was a synergistic interaction among polymorphisms in the CYP2E1, ALDH2 and ADH1B genes and heavy alcohol drinking, in Chinese males living in Gansu Province, China.

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Key words: Esophageal squamous cell carcinoma; Cytochromes P4502E1; Alcohol dehydrogenases; Aldehyde dehydrogenases; Genetic polymorphisms

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INTRODUCTION

Esophageal carcinoma is the seventh leading cause of cancer deaths worldwide[1]. It is a devastating disease with very few patients cured once diagnosed. Esophageal squamous cell carcinoma (ESCC) is one of the most...
common malignancies in Gansu province, China. There is great geographic variation in the occurrence of this tumor type in China, including exceptionally high-risk areas such as Gansu Province in the Northwest of China. Within high-risk regions in China, there is a strong tendency toward alcohol drinking, suggesting that genetic susceptibility, in conjunction with alcohol consumption, plays a role in the etiology of ESCC.

Epidemiologic studies have demonstrated that drinking alcoholic beverages is causally related to the development of ESCC[2,3]. Genetic polymorphisms in the genes encoding cytochrome P4502E1 (CYP2E1)[4-7], aldehyde dehydrogenase-2 (ALDH2) and alcohol dehydrogenase-1B (ADH1B; previously called ADH2) affect the metabolism of alcohol[8-12]. There have been some studies on the roles of alcohol and polymorphisms in the CYP2E1, ALDH2 and ADH2 genes in ESCC[13-15]. However, their results were conflicting.

To provide further data on this issue, we evaluated the susceptibility to ESCC conferred by CYP2E1, ALDH2 and ADH1B genetic polymorphisms, and defined the individual and combined roles of these genes and alcohol consumption in a high risk area for ESCC in Chinese males.

MATERIALS AND METHODS

The case participants in this study were 80 Gansu males with ESCC, treated at the Department of Gastroenterology, First Hospital of Lanzhou University and the Department of Gastroenterology, Affiliated Hospital of Gansu College of Traditional Chinese Medicine. All were registered between September 2004 and March 2007.

The 480 age-and-gender matching controls consisted of cancer-free men who received annual health checkups at two Lanzhou clinics between September 2004 and March 2007. Gansu was the ancestral home for all.

Each participant independently completed a structured questionnaire concerning his alcohol drinking habits, and those with cancer were instructed to report their habits before they were diagnosed with cancer. Each participant was asked to classify himself as a drinker or non-drinker, and to report alcohol intake as the frequency of consumption in a high-risk area for ESCC in Chinese males.

The cancer cases were age-and-gender matched with the cancer-free control subjects. The mean age of the patients was 60.2 ± 8.9, ranging from 49 to 75 years of age. The mean age of the controls was 59.7 ± 9.7, ranging from 49 to 75 years of age. The case participants in this study were 80 Gansu males containing 0.3 mol/L of MgCl₂, 5 µL of 10 × buffer, and 2 U of Taq polymerase. Briefly, the samples were denatured at 94°C for 2 min and submitted to 40 cycles of 1 min at 94°C (denaturation), 50 s at 50°C (annealing) and 50 s at 72°C (extension), with a final extension at 72°C for 10 min. PCR products (15 µL) were digested by Pst I or Rsa I restriction enzymes (1 µL of a 10 U/µL preparation) for 18 h at 37°C. Fragments were separated on 40 g/L low melting point agarose gels, and stained with ethidium bromide.

ALDH2 and ADH1B[11] polymorphisms were determined by PCR-RFLP as previously described. Each PCR analysis was performed twice, double blindly.

The allele frequency was determined by direct counting. Deviation of the genotype distribution from Hardy-Weinberg equilibrium was analyzed by the exact test. Fisher's exact test was used for comparing group statistics. The Spearman rank-correlation analysis was used as a nonparametric test for trend. All P-values were obtained from 2-sided tests. Associations between genotypes or other potential risk factors and ESCC are expressed as odds ratios (ORs) with 95% confidence intervals (CIs) adjusted for the effects of several possible confounders using a multiple logistic regression model and the STEPWISE method.

RESULTS

Five hundred and sixty males were enrolled in this study. The cancer cases were age-and-gender matched with cancer-free control subjects. The mean age of the patients was 60.2 ± 8.9, ranging from 49 to 75 years of age. The mean age of the controls was 59.7 ± 9.7, ranging from 49 to 73 years of age.

| Table 1 | Polymorphisms in the CYP2E1, ALDH2 and ADH1B genes |
|---------|-----------------------------------------------|
| Locus /protein | Gene | Subunit | Nucleotide change | Effect | RFLP |
| ADH1B | ADH1B*1 | β1 | 48G>A | Wild-type | Pst I+/Rsa I - |
| ADH1B | ADH1B*2 | β2 | 150G>A | Wild-type | Pst I-/Rsa I + |
| ALDH2 | ALDH2*1 | None | | Lys487 | |
| CYP2E1 | CYP2E1*A | | | | |
| CYP2E1 | CYP2E1*B | | | | |
| CYP2E1 | CYP2E1*1A | | | | |
| CYP2E1 | CYP2E1*2B | | | | |
| CYP2E1 | CYP2E1*1B | | | | |
| CYP2E1 | CYP2E1*2A | | | | |
| CYP2E1 | CYP2E1*1 | | | | |

CYP2E1 allele nomenclature, http://www.imm.ki.se; NIAAA publications, http://pubs.niaaa.nih.gov.

| Table 2 | Primer sequences and lengths of PCR products |
|---------|-----------------------------------------------|
| Gene | Primer | Size of PCR product (bp) | Chromosomal location |
| CYP2E1 | 5'-CCAGTCGAGTCTACATTGTCA-3' | 410 | 10q24.3-qter |
| | 5'-ATTCTGTAGATGGTGGCTGT-3' | 91 | 12q24.2 |
| ALDH2 | 5'-CCAGTCGAGTCTACATTGTCA-3' | 76 | 4q22 |
| | 5'-ATTCTGTAGATGGTGGCTGT-3' | 91 | 12q24.2 |
| ADH1B | 5'-CCAGTCGAGTCTACATTGTCA-3' | 76 | 4q22 |
| | 5'-ATTCTGTAGATGGTGGCTGT-3' | 91 | 12q24.2 |
Table 3  Alcohol drinking in esophageal cancer cases and control subjects

| Alcohol drinking | Cases ($n = 80$) | Controls ($n = 480$) | $P$ | OR 95% CI |
|------------------|-----------------|---------------------|-----|-----------|
| Status           | ($n = 80$)       | ($n = 480$)         |     |           |
| Never            | 4 (5.0)         | 132 (27.5)          | 1   | 1.01 (0.16-6.66) |
| Former           | 46 (57.5)       | 56 (7.5)            | 0.17|           |
| Current          | 20 (87.5)       | 121 (65.0)          |     | 2.22 (1.06-4.64) |
| Dose             |                 |                     |     |           |
| Non-drinker      | 4 (5.0)         | 132 (27.5)          | 1   | 1.01 (0.16-6.66) |
| Light            | 10 (12.5)       | 153 (31.9)          | 0.16| 0.68 (0.36-1.31) |
| Moderate         | 23 (28.7)       | 117 (24.4)          | 0.85| 0.54-1.07 |
| Heavy            | 43 (53.8)       | 78 (16.2)           | < 0.001| 3.20 1.32-9.65 |
| Total years of drinking |           |                     |     |           |
| Never            | 40 (50.0)       | 243 (50.6)          | 1   |           |
| $< 30$           | 17 (21.2)       | 172 (35.8)          | 0.69| 0.36-0.98 |
| $> 30$           | 23 (28.8)       | 65 (13.5)           | 0.009| 1.68 0.96-3.21 |

Table 4  Genotypes of ALDH2, ADH2 and P4502E1, n (%)

| Genotype | Cases ($n = 80$) | Controls ($n = 480$) | $P$ | OR 95% CI |
|----------|-----------------|---------------------|-----|-----------|
| ALDH2 genotype |                 |                     |     |           |
| 1/1      | 37 (46.3)       | 252 (52.5)          | 1   |           |
| 1/2      | 43 (53.7)       | 195 (40.6)          | 0.094| 2.89 1.11-5.64 |
| 2/2      | 0 (0.0)         | 33 (6.9)            |     |           |
| ADH1B genotype |                 |                     |     |           |
| 1/1      | 17 (21.3)       | 24 (5.0)            | 1   |           |
| 1/2      | 25 (31.3)       | 168 (35.0)          | < 0.001| 3.67 1.26-8.73 |
| 2/2      | 38 (47.5)       | 288 (60.0)          | < 0.001| 1.46 0.71-2.59 |
| CYP2E1 Pst I/Rsa I  |                 |                     |     |           |
| c2/c2    | 7 (8.8)         | 75 (15.6)           | 1   |           |
| c1/c2    | 16 (20.0)       | 180 (37.5)          | 0.918|           |
| c1/c1    | 57 (71.3)       | 225 (46.9)          | 0.014| 2.82 1.23-5.55 |

Table 5  Probability ratios for the combinations of ALDH2, ADH2 and CYP2E1 genotypes and the amount of alcohol consumed, n (%)

| Alcohol drinking | ALDH2 | ADH2 | CYP2E1 | OR 95% CI |
|------------------|-------|------|--------|-----------|
| Never/rare       | 1/1   | 7 (8.8) | 72 (15.0) | 1 (1.0) |
| -to-light        | 1/2   | 7 (8.8) | 180 (37.5) | 0.56 (0.20-1.59) |
|                  | 2/2   | 0 (0.0) | 33 (6.9) | 0.00 (NC) |
| Moderate         | 1/1   | 30 (37.5) | 180 (37.5) | 2.29 (0.94-5.57) |
| -to-heavy        | 1/2   | 36 (45.0) | 15 (3.1) | 8.58 (3.28-22.68) |
|                  | 2/2   | 6 (8.8)  | 42 (8.7) | 1.98 (0.98-4.0) |

Table 4 shows alcohol drinking in esophageal cancer cases and control subjects. We observed that, compared with controls, cases had a greater prevalence of heavier alcohol consumption (53.8% vs 16.2%) and a higher proportion of alcohol drinkers with $> 30$ drink-years (28.8% vs 13.5%). Heavier alcohol consumption and alcohol drinking with $> 30$ drink-years increased the risk of ESCC, with ORs (95% CI) of 3.20 (1.32-9.65) and 1.68 (0.96-3.21).

Table 4 shows the distributions of ALDH2, ADH2 and CYP2E1 genotypes. Cases and controls differed significantly in the distributions of these genotypes. These genotypes significantly deviated from the Hardy-Weinberg equilibrium (HWE) in ESCC cases, but among controls, all genotypes were in HWE.

There are two ALDH2 alleles ($^*$1 and $^*$2) and three genotypes: $^*$1/$^*$1 (GG, typical homozygote), $^*$1/$^*$2 (GA, heterozygote) and $^*$2/$^*$2 (AA, atypical homozygote), and the distributions of these genotypes were significantly different between the esophageal cancer group and the control group ($\chi^2 = 2.89, P < 0.1$). The prevalence of the inactive ALDH2 encoded by $^*$1/$^*$1 was correlated with susceptibility to esophageal cancer, with an OR (95% CI) of 2.89 (1.11-5.64).

There are three ADH1B genotypes: The wild-type genotype ($^*$1/$^*$1), heterozygote ($^*$1/$^*$2) and homozygote ($^*$2/$^*$2) genotypes. The prevalence of the less-active ADH1B encoded by $^*$1/$^*$1 increase the risk of esophageal cancer ($\chi^2 = 18.664, P < 0.0001$), OR (95% CI) of 3.67 (1.26-8.73).

There are three CYP2E1 genotypes: wild homozygote ($^*$1/$^*$1), heterozygote ($^*$1/$^*$2) and mutated homozygote ($^*$2/$^*$2) genotypes. A significant difference in the distributions of the three Pst I/Rsa I genotypes of CYP2E1 was found between the esophageal cancer group and the control group ($\chi^2 = 5.977, P < 0.05$). The c1/c1 genotype was correlated with susceptibility to esophageal cancer, with an OR (95% CI) of 2.82 (1.23-5.55).

Tables 5 and 6 show the frequency distributions and ORs for each combination of alcohol drinking habits and ALDH2, ADH1B and CYP2E1 genotypes.

The risk of ESCC was 8.58-fold higher in moderate-to-heavy drinkers with inactive ALDH2 (encoded by
ALDH2 \(*1/*1\) among never/rare-to-light \((c1/*c1)\) genes metabolizes ethanol to acetaldehyde and \((*1/*2)\) and genes ALDH2 can be considered to induce DNA damage and result in the risk of ESCC. Alcohol consumption contributes to the etiology of ESCC.

In the present study, we examined the associations of ESCC with ALDH2, ADH1B and CYP2E1 genetic polymorphisms in conjunction with alcohol drinking habits among a population at high risk of esophageal cancer. The study was conducted in Gansu province, an area of China with a relatively high alcohol consumption rate.

We found that alcohol intake was associated with ESCC, and that polymorphisms in CYP2E1, as well as in the genes encoding alcohol and aldehyde dehydrogenases \((ADH1B\) and \(ALDH2\)) are important risk factors for ESCC in Chinese men living in this high risk area.

We found that heavier alcohol consumption and alcohol drinking for \(> 30\) drink-years could increase the risk of ESCC, with ORs \((95\% CI)\) of 3.20 \((1.32-9.65)\) and 1.68 \((0.96-3.21)\), respectively. There is substantial evidence that drinking alcohol increases the risk of ESCC. Alcohol can be considered to induce DNA damage and result in the modification of nucleotides. Our risk estimates were consistent with those of previous studies. This study confirms that alcohol consumption contributes to the etiology of ESCC.

Discussing the role of certain enzymes in the metabolism of alcohol, we highlight the importance of ALDH2, ADH1B, and CYP2E1. ALDH2 is primarily responsible for the metabolism of alcohol and its derivatives, playing a key role in reducing acetaldehyde, a reactive intermediate that may initiate oxidative stress and DNA damage.

CYP2E1 is a cytochrome P450 enzyme that can activate many low molecular weight carcinogens, including certain nitrosamines, which may be involved in carcinogenesis. Alcohol metabolism and the role of CYP2E1 in the production of reactive oxygen species (ROS) and reactive free radicals are crucial in understanding the mechanisms of action of alcohol and its association with esophageal cancer.

In summary, the study demonstrates a synergistic interaction between ALDH2 \(*1/*1\) and ADH1B \(*1/*1\) and CYP2E1 \(*c1/*c2\) and \(*c2/*c2\), particularly among moderate-to-heavy drinkers, where the risk of developing ESCC is increased to a 6.82-fold higher risk than that observed in never/rare-to-light drinkers with an active \(ALDH2\) \((c1/*c1)\) genotype. This high-risk group showed a 3.12-fold higher risk \((1.86-6.58)\) of developing esophageal cancer, as compared to a 1.32-fold higher risk \((1.86-6.58)\) among moderate-to-heavy drinkers with an inactive \(ALDH2\) \((c1/*c2\) and \(*c2/*c2\)) genotype.
found that individuals with a combined ALDH2 (*1/*2) and ADH1B (*1/*1) genotype showed a dramatically increased risk of ESCC, with an OR (95% CI) of 7.46 (3.28-18.32), which is higher than those due to the respective genotypes. These findings indicate the ALDH2 (*1/*2) genotype has synergistic interactions with the ADH1B (*1/*1) genotype, contributing to the development of ESCC. Our study confirmed the findings of Tetsuji.[8]

The significant finding in this study was the interaction between the CYP2E1 and ALDH2 genotypes and heavy alcohol drinking, using a case-control study design. Previous studies have not examined this issue in detail and, to our knowledge, this is the first study to show a significant interaction between the CYP2E1 and ALDH2 genotypes and alcohol drinking. In our study, we found synergistic interactions among polymorphisms in the CYP2E1 and ALDH2 genotypes and heavy alcohol drinking; individuals with a combined ALDH2 (*1/*2) and CYP2E1 (*c1/*c1) genotype showed a dramatically increased risk of ESCC, with an OR (95% CI) of 6.82 (1.44-9.76), which is higher than those due to the respective genotypes.

Conflicting results from studies[25-28] in different countries and areas show the complexity of the biological mechanisms underlying ESCC. The susceptibility to ESCC may be correlated with genes, environment, area, race or other factors. The results of this study demonstrate that CYP2E1 (*c1/*c1), ALDH2 (*1/*2) and ADH1B (*1/*1) genotypes are associated with esophageal cancer risk among moderate-to-heavy drinking Chinese males in Gansu province. In addition, this study also showed ALDH2 (*1/*2) and CYP2E1 (*c1/*c1) carriers, and ALDH2 (*1/*2) and ADH1B (*1/*1) genotypes have a much higher risk of developing esophageal cancer, especially among alcohol drinkers. Future studies are needed to examine the biological mechanisms involved, and to evaluate the contribution of gene and environment interactions to the risk of ESCC.

## Comments

### Background

Worldwide, cancer of the esophagus ranks among the 10 most common cancers. Epidemiological studies have demonstrated that drinking alcoholic beverages is causally related to the development of esophageal squamous cell carcinoma (ESCC). Genetic polymorphisms in the P4502E1 (CYP2E1), ALDH2 and ADH1B genes affect the metabolism of alcohol. There have been some studies on the roles of alcohol and the CYP2E1, ALDH2 and ADH1B genes in ESCC. However, their results were conflicting. Therefore, the aim of the present study was to evaluate the susceptibility to ESCC conferred by CYP2E1, ALDH2 and ADH1B genetic polymorphisms, and to define the individual and combined roles of these genes and alcohol consumption in a high risk area for ESCC, among Chinese males.

### Research Frontiers

Accumulating evidence from prior epidemiologic studies suggests an association between esophageal cancer and the use of alcohol. The genetic polymorphisms of alcohol and aldehyde dehydrogenases affect the metabolism of alcohol. ALDH2 is the key enzyme involved in the elimination of acetaldehyde. Polymorphisms in the ADH1B and ALDH2 genes are associated with the risk of esophageal cancer.

### Innovations and Breakthroughs

To our knowledge, this is the first study to show significant interactions among the CYP2E1 and ALDH2 genotypes and alcohol drinking. We found synergistic interactions among polymorphisms in the CYP2E1 and ALDH2 genes and heavy alcohol drinking: Individuals with a combined ALDH2 (*1/*2) and CYP2E1 (*c1/*c1) genotype showed a dramatically increased risk of ESCC, which is higher than the risk of ESCC due to the respective genotypes.

### Applications

The detection of ALDH2, ADH1B and CYP2E1 genotypes may become a useful index for esophageal cancer, and also help clinicians to diagnose esophageal cancer earlier.

### Terminology

Esophageal squamous cell carcinoma: The most common types of esophageal cancer are squamous cell carcinoma and adenocarcinoma. Squamous cell carcinoma develops in flat cells that line the esophagus. Approximately 60% of squamous cell carcinomas develop in the middle third of the organ, 30% occur in the lower third, and 10% occur in the upper third. Adenocarcinoma develops in the lining of the esophagus and is associated with a condition called Barrett’s esophagus. This type usually occurs in the lower third of the esophagus. Genetic polymorphisms: The occurrence together in the same population of more than one allele or genetic marker at the same locus, with the least frequent allele or marker occurring more frequently than can be accounted for by mutation alone. Genetic polymorphisms provide us with the ability to predict inter-individual differences in susceptibility to clinical disease. Biomarkers of susceptibility include polymorphisms in carcinogen metabolism, DNA repair capacity and genes that control cell growth.

### Peer Review

This is an interesting study on the etiology of esophageal squamous cell cancer in China. They confirm a synergy between alcohol consumption and the phenotype of inactive enzymes.

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