DNA polymorphism and risk of esophageal squamous cell carcinoma in a population of North Xinjiang, China

Wen-Jing Ma, Guo-Dong Lv, Shu-Tao Zheng, Cong-Gai Huang, Qing Liu, Xing Wang, Ren-Yong Lin, Ilyar Sheyhidin, Xiao-Mei Lu

AIM: To investigate the role of metabolic enzyme and DNA repair genes in susceptibility of esophageal squamous cell carcinoma (ESCC).

METHODS: A case-control study was designed with 454 samples from 128 ESCC patients and 326 gender, age and ethnicity-matched control subjects. Genotypes of 69 single nucleotide polymorphisms (SNPs) of metabolic enzyme (aldehyde dehydrogenase-2, ALDH2; alcohol dehydrogenase-1 B, ADHB1; Cytochrome P450 2A6, CYP2A6) and DNA repair capacity genes (excision repair cross complementing group 1, ERCC1; O2-methylguanine DNA methyltransferase, MGMT; xeroderma pigmentosum group A, XPA; xeroderma pigmentosum group A, XPD) were determined by the Sequenom MassARRAY system, and results were analyzed using unconditional logistic regression adjusted for age, gender.

RESULTS: There was no association between the variation in the ERCC1, XPA, ADHB1 genes and ESCC risk. Increased risk of ESCC was suggested in ALDH2 for frequency of presence C allele of SNP [Rs886205: 1.626 (1.158-2.284)], XPD for C allele [Rs50872: 1.482 (1.058-2.074)], and MGMT for A allele [Rs11016897: 1.666 (1.245-2.228)]. Five variants of MGMT were associated with a protective effect on ESCC carcinogenesis, including C allele [Rs7069143: 0.698 (0.518-0.939)], C allele [Rs3793909: 0.653 (0.429-0.995)], A allele [Rs12771882: 0.618 (0.506-0.910)]. At the genotype level, increased risk of ESCC carcinogenesis was found in homozygous carriers of the ALDH2 Rs886205 [CC vs TT, odds ratios (OR): 3.116, 95% CI: 1.179-8.234], MGMT for A allele [Rs11016897: 1.666 (1.245-2.228)]. Five variants of MGMT were associated with a protective effect on ESCC carcinogenesis, carriers of the MGMT Rs11016879 (AA vs GG, OR: 3.122, 95% CI: 1.565-6.181), Rs12771882 (AA vs GG, OR: 2.442, 95% CI: 1.204-4.595), and heterozygotes carriers of the ALDH2 Rs886205 (CT vs TT, OR: 3.930, 95% CI: 1.470-10.504), MGMT Rs11016879 (AG vs GG, OR: 3.933, 95% CI: 2.216-6.982) and Rs705748 (CT vs CC, OR: 1.494, 95% CI: 1.134-3.350), respectively. Three variants were associated with a protective effect on ESCC carcinogenesis, carriers of the MGMT Rs11016879 (AA vs GG, OR: 3.933, 95% CI: 2.216-6.982) and Rs705748 (CT vs CC, OR: 1.494, 95% CI: 1.134-3.350), respectively. Three variants were associated with increased risk of ESCC metastasis in Mg/Ca for frequency of presence C allele [Rs7068306: 2.204 (1.244-3.906)], A allele [Rs10734088: 1.968 (1.111-3.484)] and C allele [Rs705748: 2.204 (1.244-3.906)].

RESULTS: There was no association between the variation in the ERCC1, XPA, ADHB1 genes and ESCC risk. Increased risk of ESCC was suggested in ALDH2 for frequency of presence C allele of SNP [Rs886205: 1.626 (1.158-2.284)], XPD for C allele [Rs50872: 1.482 (1.058-2.074)], and MGMT for A allele [Rs11016897: 1.666 (1.245-2.228)]. Five variants of MGMT were associated with a protective effect on ESCC carcinogenesis, including C allele [Rs7069143: 0.698 (0.518-0.939)], C allele [Rs3793909: 0.653 (0.429-0.995)], A allele [Rs12771882: 0.618 (0.506-0.910)]. At the genotype level, increased risk of ESCC carcinogenesis was found in homozygous carriers of the ALDH2 Rs886205 [CC vs TT, odds ratios (OR): 3.116, 95% CI: 1.179-8.234], MGMT for A allele [Rs11016897: 1.666 (1.245-2.228)]. Five variants of MGMT were associated with a protective effect on ESCC carcinogenesis, carriers of the MGMT Rs11016879 (AA vs GG, OR: 3.122, 95% CI: 1.565-6.181), Rs12771882 (AA vs GG, OR: 2.442, 95% CI: 1.204-4.595), and heterozygotes carriers of the ALDH2 Rs886205 (CT vs TT, OR: 3.930, 95% CI: 1.470-10.504), MGMT Rs11016879 (AG vs GG, OR: 3.933, 95% CI: 2.216-6.982) and Rs705748 (CT vs CC, OR: 1.494, 95% CI: 1.134-3.350), respectively. Three variants were associated with a protective effect on ESCC carcinogenesis, carriers of the MGMT Rs11016879 (AA vs GG, OR: 3.933, 95% CI: 2.216-6.982) and Rs705748 (CT vs CC, OR: 1.494, 95% CI: 1.134-3.350), respectively. Three variants were associated with increased risk of ESCC metastasis in Mg/Ca for frequency of presence C allele [Rs7068306: 2.204 (1.244-3.906)], A allele [Rs10734088: 1.968 (1.111-3.484)] and C allele [Rs705748: 2.204 (1.244-3.906)].
(1.251-3.791)]. Two variants in frequency of presence C allele of *CYP2A6* [Rs8192720: 0.290 (0.099-0.855)] and A allele of *MGMT* [Rs2053139: 0.511 (0.289-0.903)] were associated with a protective effect on ESCC progression. Increased risk of ESCC metastasis was found in heterozygote carriers of the *MGMT* Rs7068306 (CG vs CC, OR: 4.706, 95% CI: 1.872-11.833).

**CONCLUSION:** Polymorphic variation in *ALDH2*, *XPD* and *MGMT* genes may be of importance for ESCC susceptibility. Polymorphic variation in *CYP2A6* and *MGMT* are associated with ESCC metastasis.

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**Key words:** Esophageal cancer; Metabolic enzyme gene; DNA repair gene; Carcinogenesis; Metastasis

**Peer reviewers:** En-Min Li, PhD, Professor, Department of Biochemistry and Molecular Biology, Medical College, Shantou University, 22nd Xinling Road, Shantou 515041, Guangdong Province, China; Dr. Thomas Wex, PhD, Clinic of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University Magdeburg, Leipziger Str. 44, 39120 Magdeburg, Germany

Ma WJ, Lv GD, Zheng ST, Huang CG, Liu Q, Wang X, Lin RY, Sheyhidin I, Lu XM. DNA polymorphism and risk of esophageal squamous cell carcinoma in a population of North Xinjiang, China. World J Gastroenterol 2010; 16(5): 641-647 Available from: URL: http://www.wjgnet.com/1007-9327/full/v16/i5/641.htm DOI: http://dx.doi.org/10.3748/wjg.v16.i5.641

**INTRODUCTION**

Esophageal cancer (EC) is one of the most common malignancies throughout the world, ranking the eighth in incidence and the sixth in mortality among tumors of all sites[1]. However, the incidence of EC varies extremely among different regions[1,2]. The high-risk areas for esophageal squamous cell carcinoma (ESCC) (with an incidence ranging from 15 to 150/100 000) included distinct areas of South America, and the so-called “Asian Esophageal Cancer Belt” from eastern Turkey, through Iraq, Iran and the southern former Soviet Union (Kazakhstan, Turkmenistan, Uzbekistan, Tajikistan) to Mongolia and Western/Northern China[3]. Residents in the Asian EC Belt have a 10-100 fold greater chance suffering from EC than those living in the neighboring areas. ESCC is the most frequent type of EC in Asia. More than half of all ESCC cases in the world occurred in China, in the southern parts of the Taihang mountains at the borders of Henan, Shanxi and Hebei Provinces (Lin xian/Linzhou and Anyang County in Henan, and Cixian in Hebei, which will be designated below as “Linxian area”), in northern Jiangsu (Huai’an county) and in northern Xinjiang (with age standardized rates of 90-150/100000)[4,5].

Epidemiological studies revealed that the incidence of ESCC is associated with environmental factors, such as tobacco smoking, alcohol consumption, exposure to Nitrosamines, and nutritional deficiencies[6-8]. However, since only a fraction of individuals exposed to these risk factors actually develop EC, the role played by genetic determinants in response to environmental exposures needs to be addressed.

Data have also identified chronic alcohol consumption as a significant risk factor for alimentary tract cancer[9]. Ethanol is almost totally broken down by oxidative metabolism in vivo. Individuals who accumulate acetaldehyde due to polymorphic differences in the genes encoding the enzymes responsible for acetaldehyde generation and detoxification have been thought to show an ethanol associated carcinogenesis.

Ethanol is first metabolized into acetaldehyde through several enzymatic and nonenzymatic mechanisms, the main enzymatic pathways being alcohol dehydrogenase, cytochrome P450 (*CYP*) and catalase. Most of the acetaldehydes generated during alcohol metabolism in vivo are promptly eliminated by aldehyde dehydrogenase-2 (*ALDH2*), and alcohol dehydrogenase-1B (*ADH1B*, previously called *ADH2*)[10,11].

Cytochrome P450 is a Phase 1 enzyme responsible for activating most environmental pre-carcinogens, whereas glutathione S-transferases (*GSTs*) are Phase II enzymes capable of detoxifying the electrophile carcinogens that result from the action of *CYP* enzymes. *CYP2A6*, the main enzymes capable of activating nitrosamines and other carcinogens in humans, are expressed in esophageal mucosa of Brazilian patients, with a high degree of variation in expression[12]. The *CYP2A6* gene is located on chromosome 19, and 30 different alleles have been described[13].

DNA repairing capacity (DRC) is an essential component in EC progression. Sufficient DNA repair activity ensures the stability and fidelity of the genome when exposed to carcinogens in the process of cell growth and differentiation[14,15], whereas instability in the genome in cancer patients may indicate a possible involvement of defective DRC[16]. DNA repair processes generally consist of direct reversal (DR), base excision repair, nucleotide excision repair (NER), or mismatch repair pathways[17]. Each pathway is specific for the repair of one or more types of DNA damage.

Key proteins in the transcription-coupled NER pathway (involved in correcting UV-induced lesions, chemical adducts, and crosslinks) include xeroderma pigmentosum group A and D (*XPA* and *XPD*), and excision repair cross complementing group 1 (*ERCC1*)[17,18].

*O*-methylguanine DNA methyltransferase (*MGMT*) is a single protein responsible for the DR pathway. The major defense against alkylating mutations is from *MGMT*, a 207 amino acid DNA repair protein that transfers potentially carcinogenic alkylating adducts from the DNA to a cysteine residue of *MGMT[19,20]*. For each adduct removed, an *MGMT* molecule is inactivated, hence the capacity for each cell to repair DNA depends upon the total number of *MGMT* molecules in the cell.
When genes essential for a variety of DNA repair pathways display aberrant activities, this may influence DRC with consequent carcinogenesis and progression. Several studies suggested that reduced expression of certain DNA repair genes were associated with the risk of environment-related esophageal adenocarcinoma[21-23]. There are few studies that examined the roles of gene polymorphism, metabolic enzyme and several DNA repair genes in risk of ESCC.

In the present study, Sequenom MassARRAY system was utilized to determine the relationship between ESCC and seven genes (ADHB1, ALDH2, ERCC1, MGMT, XP4, XPD, and CYP2A6) involved in two different DNA damage and repair pathways. The relationship between the polymorphism of candidate DNA repair genes and ESCC was determined in peripheral blood mononuclear cells from 128 patients with newly diagnosed, untreated ESCC and 326 healthy controls from the northern Xinjiang.

MATERIALS AND METHODS

Study subjects
This study included 128 ESCC patients and 326 healthy controls. All cases were from the Northern Xinjiang, China. Patients were newly diagnosed with histologically confirmed primary ESCC and not treated with radiotherapy or chemotherapy previously from January 2006 to December 2008. Healthy control subjects were recruited from a cancer-screening program for early detection of EC in the same area and matched with ESCC patients on age (± 5 years), gender, ethnicity and residence. The selection criteria included no individual history of cancer and digestive disease. After written consent of blood donation for research purposes was obtained from cases and control, each subject donated 5 mL peripheral blood collected in K3EDTA tubes and stored at -80°C until DNA was extracted.

Single nucleotide polymorphism (SNP) selection
The International Haplotype Mapping (www.hapmap.org) and Cancer SNP databases (http://snp500cancer.ncbi.nih.gov/snpcfm) were used to select SNPs in seven genes. The selection criteria was that SNPs had an r² of ≥ 0.8 for all SNPs with a minor allele frequency > 5% in CHB. According to the above rules, 69 Tag SNPs were selected in seven genes, including 6 for XP4, 5 for XP4, 5 for ERCC1, 6 for ALDH2, 5 for ADHB1, 5 for XYP2A6, and 41 for MGMT.

Interview
A trained interviewer administered a questionnaire to cases and control, gathering clinical and demographical information. The questions included demographic variables (age, gender, and ethnicity), and a comprehensive smoking and alcohol intake profile. Smoking habits, and alcohol status were all defined at 1 year prior to diagnosis for cases, or 1 year prior to interview for controls. Smoking of the patients was classified as never-smokers, ex-smokers (quit > 1 year), and current smokers (smoking currently or quit < 1 year). Alcohol use was defined as patients who never consumed alcohol and those who had consumed alcohol at any time in the past.

Genotyping
Genomic DNA was extracted within 1 wk after sampling using proteinase K digestion followed by a salting out procedure according to the method published by Miller et al[24]. SNPs were genotyped using the Sequenom MassARRAY system. The i-PLEX assay was performed according to manufacturer's instructions (www.sequenom.com) using 5 ng of genomic DNA.

SNP typing was conducted using the Sequenom™ iPLEX™ protocol. PCR reactions were performed in standard 384-well plates containing 10 ng genomic DNA, 0.5 U of Taq polymerase (HotStarTaq, Qiagen, CA), 500 μmol of dNTPs and 100 nmol of both forward and reverse PCR primers. Thermocycling conditions within the ABI-9700 (Applied Biosystems, USA) consisted of an initial 15 min denaturation at 94°C, followed by 45 cycles of 20 s denaturing at 94°C, 30 s annealing at 56°C, and 60 s extension at 72°C. PCR products were purified by incubation at 37 using 0.15 U of Shrimp Alkaline Phosphatase for 30 min followed by a 5 min inactivation at 85°C. A primer extension reaction mixture including 0.1 μL of a 10 × termination mix, 0.02 μL DNA polymerase and 1000 nmol/L of the extension primers was used in both the initial (denaturation at 94°C for 30 s, followed by 5 annealing and extension cycles at 52 and 80°C, respectively) and secondary (40 cycles of 5 s denaturation at 94°C, 5 s annealing at 52°C and 5 s extension at 80°C) iPLEX reactions. A final extension for 3 min at 72°C was conducted prior to cooling at 20°C. Products were diluted and desalted with 15 μL sterile water and 3 μL of resin prior to spotting onto a SpectroChip for analysis in the Compact Mass Spectrometer, using Workstation software version TYPER 4.0 (Sequenom). Genotype accuracy was calculated for all SNPs tested at 99.95%.

For quality control, genotyping was repeated randomly in at least 5% of the samples, and two of the authors independently reviewed all results. Allele frequencies of controls were calculated using the formula (example genotypes AA, AB and BB): Allele B frequency = [number of genotypes AB + 2 × (number of genotypes BB)]/[2 × (number of genotypes AA + number of genotypes AB + number of genotypes BB)].

Statistical analysis
All cases and controls were compared for age, gender and ethnicity to ensure frequency matching. Hardy-Weinberg equilibrium of allele distributions was tested in cases and controls, separately. We used unconditional multivariate logistic regression to assess the main effects of genetic polymorphisms on ESCC risk by estimating odds ratios (OR) and associated confidence intervals (CI). Genotypes were categorized into three groups when the allele frequencies were allowed (major allele homozygous, heterozygous and homozygous variants).
### RESULTS

#### Subject characteristics

The frequency of 69 sequence variants was assessed in DNA samples from 128 ESCC cases and 326 controls from the population of North-Western China. The distributions of age and gender, smoking history, alcohol consumption history, and pathological grade among the study subjects are summarized in Table 1. There were no statistically significant differences among cases and controls in terms of mean age, gender and ethnicity, suggesting that the frequency matching was adequate.

As expected, the prevalence of smoking and alcohol was both significantly higher in ESCC cases than in the controls (Table 1).

There was a greater proportion of ex-smokers and current-smokers in the cases than in the controls (P = 0.007). The percentage of ever alcohol users in the cases was higher than in the controls (P = 0.002). There were 36 (28.1%) cases with metastasis and 92 (71.9%) cases without metastasis.

#### Analysis of seven genes genotype in ESCC carcinogenesis

At the genotype level, increased risk of ESCC was found in homozygous carriers of the *ALDH2* Rs886205 (CC vs TT, OR: 3.116, 95% CI: 1.179-8.234), *MGMT* Rs11016879 (AA vs GG, OR: 3.116, 95% CI: 1.565-6.181) and Rs12771882 (AA vs GG, OR: 2.442, 95% CI: 1.204-4.595). Increased risk of ESCC was also observed in heterozygotes carriers of the *ALDH2* Rs886205 (CT vs TT, OR: 3.930, 95% CI: 1.470-10.504), *MGMT* Rs11016879 (AG vs GG, OR: 3.933, 95% CI: 2.16-6.982) and Rs7075748 (CT vs CC, OR: 1.949, 95% CI: 1.134-3.350), respectively.

Three variants were associated with a protective effect on ESCC carcinogenesis: the carriers of the *MGMT* Rs11016879 (AG vs AA, OR: 0.388, 95% CI: 0.180-0.836), Rs7069143 (CT vs CC, OR: 0.478, 95% CI: 0.303-0.754) and Rs7071825 (GG vs AA, OR: 0.493, 95% CI: 0.266-0.915).

#### Analysis of seven genes allele in ESCC progression

Increased risk of ESCC metastasis was found in heterozygote carriers of the *MGMT* Rs 7068306 (GG vs CC, OR: 4.706, 95% CI: 1.872-11.833).

### DISCUSSION

ESCC is one of the major health issues in China because of its high incidence and poor survival. Although the exact mechanism on EC is unclear, several possible mechanistic pathways have been proposed, including metabolic enzyme and DNA repair factors.

Alcohol consumption and tobacco smoking are established major risk factors for ESCC in Western populations. However, studies in high-incidence regions are scarce and their results have been inconsistent. In the present study, we first conducted a case-control study to evaluate the associations between metabolic enzyme polymorphisms of *ADH1B*, *ALDH2* and *CYP2A6* and ESCC risk in population from northern Xinjiang, China. Consistent with previous studies, the present study demonstrated that alcohol consumption and cigarette smoking are strongly associated with the incidence of ESCC. Moreover, individuals with *ALDH2* for frequency of presence C allele of SNP in Rs886205 (CT vs TT) had significantly increased risk for ESCC carcinogenesis while individuals with frequency of

| Table 1 Characteristics of ESCC patients and controls (n (%) |
|-----------------|----------------|
| Variables       | Controls (n = 326) | Patients (n = 128) | P value |
| Age (yr)        | 61 (55-78)        | 63 (58-81)         | 0.950   |
| Median (range)  | 160               | 58                 | 0.002   |
| Gender          | 166               | 70                 | 0.470   |
| Smoking status  |                  |                   |
| Non-smokers     | 112 (34.3)        | 26 (20.3)          |         |
| Ex-smokers      | 152 (46.7)        | 66 (51.6)          |         |
| Current smokers | 62 (19.0)         | 36 (28.1)          | 0.007   |
| Alcohol use     |                  |                   |
| Never           | 81 (24.9)         | 15 (11.7)          |         |
| Ever            | 245 (75.1)        | 113 (88.3)         | 0.002   |
| Metastasis      |                  |                   |
| No              | 92 (71.9)         | 92 (71.9)          |         |
| Yes             | 36 (28.1)         | 36 (28.1)          |         |
| Ethnicity       |                  |                   |
| Kazakh          | 89 (27.3)         | 39 (30.5)          |         |
| Uygur           | 87 (26.7)         | 30 (23.4)          |         |
| Han             | 150 (46.0)        | 59 (46.1)          | 0.703   |

Analysis of seven genes genotype in ESCC carcinogenesis

At the genotype level, increased risk of ESCC was found in homozygous carriers of the *ALDH2* Rs886205 (CC vs TT, OR: 3.116, 95% CI: 1.179-8.234), *MGMT* Rs11016879 (AA vs GG, OR: 3.116, 95% CI: 1.565-6.181) and Rs12771882 (AA vs GG, OR: 2.442, 95% CI: 1.204-4.595). Increased risk of ESCC was also observed in heterozygotes carriers of the *ALDH2* Rs886205 (CT vs TT, OR: 3.930, 95% CI: 1.470-10.504), *MGMT* Rs11016879 (AG vs GG, OR: 3.933, 95% CI: 2.16-6.982) and Rs7075748 (CT vs CC, OR: 1.949, 95% CI: 1.134-3.350), respectively.

Three variants were associated with a protective effect on ESCC carcinogenesis: the carriers of the *MGMT* Rs11016879 (AG vs AA, OR: 0.388, 95% CI: 0.180-0.836), Rs7069143 (CT vs CC, OR: 0.478, 95% CI: 0.303-0.754) and Rs7071825 (GG vs AA, OR: 0.493, 95% CI: 0.266-0.915).

Analysis of seven genes allele in ESCC progression

Increased risk of ESCC metastasis was found in heterozygote carriers of the *MGMT* Rs 7068306 (GG vs CC, OR: 4.706, 95% CI: 1.872-11.833).
Table 2  Analysis of genes allele in ESCC carcinogenesis

| Gene | Locus       | Control allele frequency (n = 326) | Case allele frequency (n = 128) | OR (95% CI)   | P value |
|------|-------------|-----------------------------------|-------------------------------|---------------|---------|
|      |             | 1       | 2        |                |         |
| ALDH2 | Rs886205   | 448     | 204      |                 |         |
| XPD  | Rs50872    | 522     | 130      |                 |         |
| MGMT | Rs7069143  | 356     | 296      |                 |         |
| MGMT | Rs3793909  | 535     | 117      |                 |         |
| MGMT | Rs12771882 | 165     | 487      |                 |         |
| MGMT | Rs551491   | 280     | 372      |                 |         |
| MGMT | Rs11016879 | 264     | 388      |                 |         |
| MGMT | Rs7071825  | 415     | 237      |                 |         |

Table 3  Analysis of genes genotype in ESCC carcinogenesis

| Gene/ genotype | Control (n = 326) | Cases (n = 128) | OR (95% CI)   | P value |
|----------------|-------------------|-----------------|---------------|---------|
| ALDH2          |                    |                 |               |         |
| TT             | 40 (12.270)       | 5 (3.906)       | 1.0 (reference) |         |
| CT             | 114 (34.969)      | 56 (43.750)     | 3.950 (1.470-10.504) | 0.004 |
| CC             | 172 (52.761)      | 67 (52.344)     | 3.116 (1.179-8.234) | 0.017 |
| MGMT           | Rs11016879        |                 |               |         |
| AA             | 18 (5.522)        | 15 (11.719)     | 1.0 (reference) |         |
| AG             | 130 (39.877)      | 42 (32.815)     | 0.388 (0.180-0.836) | 0.013 |
| GG             | 78 (24.601)       | 71 (55.466)     | 1.281 (0.992-1.614) | 0.047 |
| MGMT           | Rs7069143         |                 |               |         |
| CC             | 87 (26.687)       | 54 (42.188)     | 1.0 (reference) |         |
| CT             | 182 (55.828)      | 54 (42.188)     | 0.478 (0.303-0.754) | 0.001 |
| TT             | 27 (8.478)        | 20 (15.625)     | 0.560 (0.306-1.043) | 0.066 |
| MGMT           | Rs12771882        |                 |               |         |
| GG             | 181 (55.521)      | 63 (49.219)     | 1.0 (reference) |         |
| AG             | 125 (38.344)      | 48 (37.500)     | 0.903 (0.711-1.172) | 0.661 |
| AA             | 20 (6.350)        | 17 (13.280)     | 2.422 (1.204-4.595) | 0.011 |
| MGMT           | Rs7075748         |                 |               |         |
| CC             | 88 (26.994)       | 42 (33.218)     | 1.0 (reference) |         |
| CT             | 156 (47.853)      | 76 (59.375)     | 1.949 (1.134-3.350) | 0.015 |
| TT             | 82 (25.350)       | 30 (23.438)     | 1.463 (0.782-2.740) | 0.233 |
| MGMT           | Rs7071825         |                 |               |         |
| AA             | 46 (14.349)       | 24 (19.231)     | 1.0 (reference) |         |
| AG             | 144 (44.375)      | 69 (56.219)     | 0.918 (0.519-1.625) | 0.770 |
| GG             | 136 (42.276)      | 35 (27.923)     | 0.493 (0.266-0.915) | 0.024 |

presence C allele in CYP2A6 of Rs8192720 were inversely associated with ESCC metastasis.

Genomic fidelity and genetic stability usually depend on the efficiency of DRC when an organism is exposed to environmental and endogenous carcinogens. Peltohämä[15] showed that aberrant DRC might be involved in the pathogenesis of some cancers.

Previous molecular epidemiological studies have found that the XPD polymorphism is associated with increased risks for head and neck cancers[34]. However, inconsistent findings were also reported, including inverse associations with esophageal adenocarcinoma[35]. Our results suggested that XPD for frequency of presence C allele of SNP in Rs50872 increased the risk of ESCC carcinogenesis, which is consistent with the reported findings[36,37].

MGMT is a major gene in the pathway of DNA repair and frequently found to be silenced by CpG island hypermethylation in many cancers, such as gastric cancer[38] and esophageal adenocarcinoma[39]. However, the studies on the ESCC are still rare. Fang et al[40] reported that 29% (5 of 17) of normal esophageal tissues, 50% (10 of 20) of basal cell hyperplasia, 67% (8 of 12) of dysplasia, and 72% (13 of 18) of ESCC samples obtained from Linzhou City of Henan Province in northern China had DNA hypermethylation in MGMT promoter region. Until now, no investigation has been carried out about the associations between MGMT polymorphism and ESCC in a population of high incidence region of Northern Xinjiang. Our findings displayed that MGMT for frequency of presence A allele of SNP in Rs11016879, carriers of MGMT Rs11016879 (AA vs GG, AG vs GG), Rs12771882 (AA vs GG), and Rs7075478 (CT vs CC) significantly increased the risk of ESCC carcinogenesis. Our results are in agreement with the previous studies on ESCC metastasis which was inconsistent with previous studies[41-43].

Furthermore, increased risk of ESCC metastasis was indicated in MGMT for frequency of presence C allele of Rs7068306, A allele in Rs10734088 and C allele in Rs751115, and carriers of MGMT Rs7068306 (GG vs CC).

In addition, variants of MGMT were associated with a protective effect on ESCC carcinogenesis, including C allele in Rs7069143, C allele in Rs3793909, A allele in Rs12771882, C allele in Rs551491, and A allele in Rs7071825, and the carriers of the MGMT in Rs11016878 (AG vs AA), Rs7069143 (CT vs CC) and Rs7071825 (GG vs AA). Frequency of C allele in Rs8192720 and A allele in Rs2053139 were associated with a protective effect on ESCC metastasis which was consistent with previous studies[44,45].

Difference in study population may be one of the reasons leading to the different results. However, since this is only a retrospective study and our findings could not be stratified because of the relatively small number in the groups, further epidemiological studies with enlarged samples are worth doing to warrant the results.

A limitation of our study may be missing covariate data. There have been many studies of individual environmental risk factors for EC[46-48]. We did not collect information on dietary factors and occupational history and so we were unable to study gene-environmental interactions in the etiology of ESCC. Gene-smoking and gene-alcohol interactions cannot be accurately measured due to sample size considerations. However, an improved
understanding of the main separate effects of genes and environment will allow detailed gene-environmental interactions to be examined in larger-scale studies in the future. Our study population size is too small to avoid spurious results although they are suggestive, and further larger validation studies are certainly needed. The biological meaning and function of interesting SNPs need to be confirmed especially in advanced researches.

However, the strong points in our study are that both cases and healthy control subjects were recruited from the same area and matched on age, gender, ethnicity and residence, and all our control subjects were under Hardy-Weinberg equilibrium. Moreover, all our cases were pathologically confirmed, and followed by a strict quality control from genotyping.

To conclude, this study provides no evidence on a role of common variation of genes ERCC1, XP-A, and ADH1B in ESCC. However, our data indicated that variation in the ALDH2, XPD, and MGMT genes may be related to the risk of ESCC carcinogenesis. The polymorphic variation in CYP2A6 and MGMT are associated with ESCC metastasis.

### COMMENTS

#### Background
Because of the high incidence and poor survival, esophageal cancer (EC) is one of the major health issues in Western/Northern China. Although the exact mechanism is unclear, several possible mechanistic pathways have been proposed, including metabolic enzyme and DNA repair factors.

#### Research frontiers
The relationship between gene polymorphism of metabolic enzyme, DNA repair and EC in Western/Northern China needs to be addressed. The present study indicated that metabolic enzyme and DNA repair factors were involved in the risk of esophageal squamous cell cancer (ESCC).

#### Innovations and breakthroughs
The variation of ALDH2, XPD, and MGMT genes may be of relevance to the risk of ESCC. The polymorphic variation of CYP2A6 and MGMT were associated with ESCC metastasis.

#### Applications
By understanding the gene polymorphism in ALDH2, CYP2A6, MGMT and XPD, further researches may represent a future strategy for detecting the susceptibility to ESCC.

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**Table 4 Analysis of genes allele in ESCC progression**

| Gene     | Locus       | Control allele frequency (n = 92) | Case allele frequency (n = 36) | OR (95% CI) | P value |
|----------|-------------|----------------------------------|-----------------------------|-------------|---------|
|          |             | 1       | 2       | 1       | 2       |             |              |
| CYP2A6   | Rs8192720   | 153    | 31      | 68      | 4       | 0.290 (0.099-0.855) | 0.018       |
| MGMT     | Rs7068306   | 137    | 47      | 41      | 31      | 2.204 (1.244-3.796) | 0.006       |
| MGMT     | Rs10734088  | 135    | 49      | 42      | 30      | 1.968 (1.113-3.484) | 0.019       |
| MGMT     | Rs4751115   | 112    | 72      | 30      | 42      | 2.178 (1.251-3.791) | 0.005       |
| MGMT     | Rs2053139   | 93     | 91      | 48      | 24      | 0.511 (0.289-0.903) | 0.020       |

**Table 5 Analysis of genes genotype in ESCC progression n (%)**

| Gene/ genotype | Without metastasis (n = 92) | With metastasis (n = 36) | OR (95% CI) | P value |
|----------------|-----------------------------|--------------------------|-------------|---------|
| MGMT Rs7068306 | CC                          | 60 (65.217)              | 12 (33.333) | 1.0 (reference) |        |
|                | CG                          | 17 (18.478)              | 16 (44.444) | 4.706 (1.872-11.833) | 0.001 |
|                | GG                          | 15 (16.304)              | 8 (22.222)  | 2.667 (0.925-7.685)  | 0.064 |

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**Peer review**

The paper deals with a very interesting subject. The authors analyzed genotypes of sixty-nine single nucleotide polymorphisms (SNPs) of metabolic enzyme and DNA repair capacity related genes in a moderate scale, and found some SNPs correlated with the risk of ESCC and tumor metastasis. These results are attractive and interesting. In general, the manuscript is written well and the data is presented clearly.
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