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ORIGINAL ARTICLE

Polymorphisms of *COMT* and *XPD* and risk of esophageal squamous cell carcinoma in a population of Yili Prefecture, in Xinjiang, China

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Abstract

Objective: To investigate polymorphisms of *COMT* (Rs4680) and *XPD* (Rs13181) and risk of esophageal squamous cell carcinoma (ESCC) in a population from Yili Prefecture, Xinjiang, China.

Methods: A hospital-based case–control study was designed. Genotypes were determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Odds ratios (OR) and 95% confidence intervals (CI) were analysed using unconditional logistic regression.

Results: An increased risk of ESCC was discovered with *COMT* in relation to the frequency of the presence of the A allele (Rs4680; OR 1.30, 95% CI 1.00–1.68). An individual with combined *COMT* 158 (Val/Met or Met/Met) and *XPD* 751 (Lys/Gln or Gln/Gln) genotype had an increased ESCC risk.

Conclusions: Polymorphic variation in *COMT* Val158Met and *XPD* Lys751Gln may be important for ESCC susceptibility.

Keywords: *Esophageal squamous cell carcinoma; COMT gene; XPD gene; polymorphisms*

Introduction

Currently, the prognosis for patients suffering from esophageal squamous cell carcinoma (ESCC) still remains poor. According to the World Health Organization, incidence spectra are located between Western Africa at the low-risk end, and China at the high-risk end, including the so-called 'Asian esophageal cancer belt' (Stewart et al. 2003). Esophageal cancer (EC) is one of the most common malignant diseases in China. The incidence of EC varies considerably between different regions (Jemal et al. 2005, Lambert & Hainaut 2007): in the southern parts of the Taihang mountains at the borders of Henan, Shanxi and Hebei Provinces (Linxian/Linzhou and Anyang County in Henan, and Cixian in Hebei, which will be designated below as 'Linxian area'), in northern Jiangsu (Huaian county) and in northern Xinjiang (with age standardized rates of 90–150/100 000) (Lu et al. 1985, Zhang 1988). The carcinogenesis and development of EC is a complex process involving multiple factors, stages and numerous changes in genes and proteins at the molecular level.

The catechol estrogen is catalysed and inactivated by catechol-*O*-methyltransferase (COMT). The accumulation of catechol estrogens has the potential to induce DNA damage and form DNA adducts, which increases the risk of carcinoma (Cavalieri et al. 1997, 2000). The *COMT* Val158Met allele encodes a thermolabile variant of the enzyme that in homozygotes confers a ~2-to 4-fold lower catalytic activity (Syvanen et al. 1997, Dawling et al. 2001). Xeroderma pigmentosum complementation group D (*XPD*) gene is one of the seven genetic complementation groups encoding for proteins involved in the nucleotide excision repair (NER) pathway (Wood 1999, Benhamou & Sarasin 2002, Manuguerra et al. 2006). Some studies have shown the *XPD* Lys751Gln polymorphism may be associated with decreased DNA repair capacity and increased tumour risk (Shen et al. 1998, Spitz et al. 2001, Affatato et al. 2004, Rzeszowska-Wolny et al. 2005, Vineis et al. 2009).

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We hypothesized that the low-activity alleles, *COMT*^{Met} or *XPD*^{Gln}, may be implicated in ESCC. Therefore, we assessed the association between these two polymorphisms (Rs4680 and Rs13181) and ESCC susceptibility in a hospital-based case–control study with a population of Yili Prefecture, in Xinjiang, China.

Materials and methods

Samples

A hospital-based case–control study was designed. The study included 566 samples, from 356 healthy controls and 210 ESCC patients. All subjects were from the Yili Prefecture, in Xinjiang, China. Patients were newly diagnosed with histologically confirmed primary ESCC and had not been treated previously with radiotherapy and chemotherapy from January 2006 to December 2008. The healthy subjects, who had no history of cancer or digestive disease, were recruited from individuals who visited the same hospital for physical examination during the same period in the same area and matched with ESCC patients by age (± 5 years), gender, ethnicity and residence.

DNA extraction

Questionnaires were completed by the ESCC patients and controls. After written consent for blood donation, blood samples (5 ml whole blood/EDTA) were collected from each subject and stored at –80°C. Genomic DNA extraction was performed by proteinase K digestion, followed by a salting out procedure, as described previously (Miller et al. 1988).

Genotype analyses

Genotyping assays for the single nucleotide polymorphisms (SNP) of *COMT* and *XPD* genes were done using polymerase chain reaction– restriction fragment length polymorphism (PCR-RFLP) methods. A 217-bp fragment of the *COMT* Val158Met gene (Kunugi et al. 1997) (G→A) was amplified using the forward primer: 5′-TCG TGG ACG CCG TGA TTC AGG -3′ and the reverse primer 5′-AGG TCT GAC AAC GGG TCA GGC-3′. Primers for the *XPD* Lys751Gln gene (Spitz et al. 2001) (A→C) (436 bp) were 5′-GCC CGC TCT GGA TTA TAC G-3′ (forward) and 5′-CTA TCA TCT CCT GGC CCC C-3′ (reverse). The 20-µl multiplex PCR mix contained 100 ng DNA 2 µl, Premix Ex Tap (TaKaRa, Shiga, Japan) 10 µl, forward or reverse primer 0.3 µl (10 pmol µl^{-1}) and ddH_2O 7.4 µl. The DNA was first denatured for 5 min at 95°C, then amplified during 35 cycles of 95°C for 30 s, 59°C for *COMT* and 61°C for *XPD* for 30 s, and 72°C for 30 s, and subsequently by a 7 min extension at 72°C. Ten microlitres of PCR product was incubated (2.5 units of *Nla*III for *COMT*) (Fermentas Company, Shenzhen City, China) or (5 units of *Pst*I for *XPD*) (TaKaRa) in a volume of 20 µl at 37°C overnight. The amplicons were separated by agarose gel electrophoresis (4% agarose gel for *COMT* or 3% agarose gel for *XPD*). The gel was stained with ethidium bromide. The DNA was visualized in UV light. Thus, fragments of homozygotes for *COMT* Val allele were 136 and 81 bp, heterozygotes showed 136, 96, 81 and 40 bp fragments, and homozygotes for *COMT* Met allele generated 96, 81 and 40 bp fragments (the 40-bp fragment in the present 4% agarose gel could not be observed) (Figure 1). *Pst*I digestion resulted in two fragments of 290 and 146 bp for the wild-type alleles (Lys/Lys), three fragments (227, 146 and 63 bp) for the variant alleles (Gln/Gln) and four fragments (290, 227, 146 and 63 bp) for the heterozygotes (Lys/Gln) (the 63-bp fragment in the present 3% agarose gel could not be observed) (Figure 2).

Allele frequencies were calculated using the formula (e.g. genotypes AA, AB and BB): allele B frequency = [number of genotypes AB + 2 × (number of

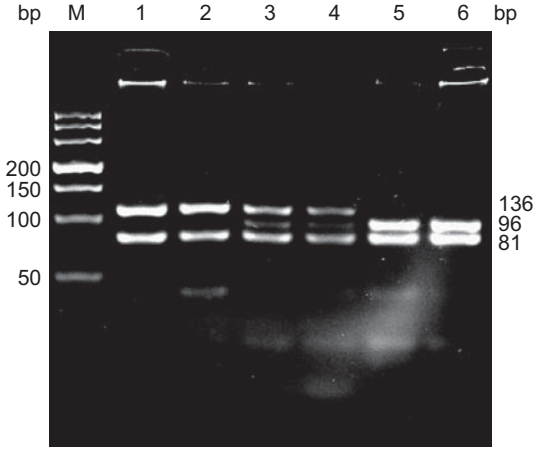

Figure 1. Electrophoresis of patient's polymerase chain reaction products containing *COMT* Val158Met digested by *Nla*III. M, DNA marker; lanes 1 and 2, homozygous wild-type (G/G); lanes 3 and 4, heterozygous type (G/A); lanes 5 and 6, homozygous variant genotype (A/A).

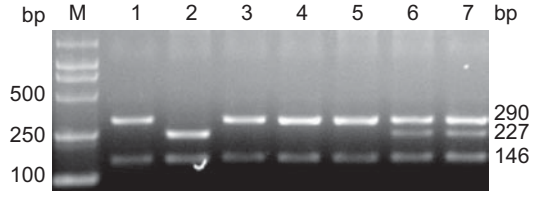

Figure 2. Electrophoresis of patient's polymerase chain reaction products containing *XPD* Lys751Gln digested by *Pst*I. M, DNA marker; lane 1, 3 and 4, homozygous wild-type (A/A); lanes 6 and 7, heterozygous type (A/C); lane 2, homozygous variant genotype (C/C).

genotypes BB)]/[2 × (number of genotypes AA + number of genotypes AB + number of genotypes BB)].

Statistical analyses

All statistical analyses were performed using SPSS version 11.5 software package (SPSS, Chicago, IL, USA). All cases and control were compared for age, gender and ethnicity to ensure frequency matching. A Hardy–Weinberg equilibrium analysis was performed to compare the observed and expected genotype frequencies using the χ^2 test. The comparison of *COMT* and *XPD* genotype, allelotype distribution in the study groups was performed by means of two-sided contingency tables using the χ^2 test. Relative risk associated with a particular genotype was estimated by calculating odds ratio (OR) along with 95% confidence interval (CI). A p-value <0.05 was considered statistically significant.

Results

Subject characteristics

A total of 356 normal controls and 210 cases of ESCC were recruited in the present study from the population of Yili Prefecture, in Xinjiang, China. The distributions of age, sex and ethnicity among cases and control are summarized in Table 1. There were no significant differences between cases and controls in terms of distributions of gender, age and ethnicity, which suggested that the frequency matching was adequate. No special history of ESCC among cases and controls was reported.

Analysis of genotypes in ESCC carcinogenesis

ESCC patients and controls were in Hardy–Weinberg equilibrium for the two genotypes analysed ($\chi^2 = 0.55$, $p = 0.76$). Genotyping results (Table 2) showed the distribution of *COMT* Val158Met genotypes among controls (Val/Val, 50.6%; Val/Met, 41.0%; Met/Met, 8.4%) and ESCC patients (42.9%, 45.2%, 11.9%). The distribution of Lys/Lys, Lys/Gln and Gln/Gln genotypes at the *XPD* Lys751Gln site among controls was 74.6%, 22.2% and 1.4%, but not significantly different among ESCC patients (70.5%, 25.7%, 3.8%). At the genotype level, there were no associations of genetic polymorphisms in *COMT* Vall58Met and *XPD* Lys751Gln with risk of ESCC in a population of Yili Prefecture, in Xinjiang, China. Neither the carriers of the *COMT* Rs4680 (GG vs AA, OR 1.68, 95% CI 0.93–3.00) nor *XPD* Rs13181 (AA vs CC, OR 2.94, 95% CI 0.95–9.15) had an increased risk of ESCC. However, we observed that individuals with the combined *COMT* 158 (Val/Met or Met/Met) genotype and *XPD* 751 (Lys/Gln or Gln/Gln) genotype had an increased ESCC risk, compared with somebody who carried wild type for both of these alleles (OR 1.72, 95% CI 1.02–2.89) (Table 3).

Table 1. Characteristics of esophageal squamous cell carcinoma patients and controls.

Variable	Cases (%)	Controls (%)	p-Value
Total	210 (37.1)	356 (62.9)	
Gender			0.13
Male	118	223	
Female	92	133	
Age (years), median (range)	64 (58–81)	62 (55–78)	0.96
Ethnicity			
Han	114 (54.3)	202 (56.7)	0.78
Uygur	45 (21.4)	68 (19.1)	
Kazakh	51 (24.3)	86 (24.2)	

Analysis of two gene alleles in ESCC carcinogenesis

Table 4 shows the two genes alleles in ESCC carcinogenesis. Increased risk of ESCC carcinogenesis was indicated in *COMT* in relation to the frequency of the presence of the A allele (OR 1.30, 95% CI 1.00–1.68).

Analysis of two gene genotypes in ESCC progression (location of tumour, histological differentiation, invasion and lymph node metastasis)

We also analysed the two genes genotype of clinicopathological parameters (location of tumour, histological differentiation, invasion and lymph node metastasis) in ESCC progression. But there was no association of genetic polymorphisms in the *COMT* Vall58Met and *XPD* Lys751Gln with ESCC progression in a population of Yili Prefecture, in Xinjiang, China (data not shown).

Table 2. Analysis of genotype in esophageal squamous cell carcinoma carcinogenesis.

Genotype	Controls, n (%)	Cases, n (%)	OR	(95% CI)	p-Value
COMT Val158Met					
Val/Val (GG)	180 (50.6)	90 (42.9)	1.0		
Val/ Met (GA)	146 (41.0)	95 (45.2)	1.30	(0.91–1.87)	0.15
Met/Met (AA)	30 (8.4)	25 (11.9)	1.68	(0.93–3.00)	0.09
Val/Val or Val/ Met	326 (91.6)	185 (88.1)	1.00		
Met/Met	30 (8.4)	25 (11.9)	1.47	(0.84–2.57)	0.18
XPD Lys751Gln					
Lys/Lys (AA)	272 (74.6)	148 (70.5)	1.0		
Lys/Gln (AC)	79 (22.2)	54 (25.7)	1.26	(0.84–1.88)	0.26
Gln/Gln (CC)	5 (1.4)	8 (3.8)	2.94	(0.95–9.15)	0.05
Lys/Lys or Lys/Gln	351 (98.6)	202 (96.2)	1.0		
Gln/Gln	5 (1.4)	8 (3.8)	2.78	(0.90–8.61)	0.07

OR, odds ratio; CI, confidence interval.

Table 3. Analysis of *COMT* and *XPD* genes genotype in esophageal squamous cell carcinoma carcinogenesis.

COMT	*XPD*	Controls	Cases	OR	95% CI	*p*-Value
Val/Val (wild type)	Lys/Lys (wild type)	145	65	1.0		
Val/Met+Met/Met	Lys/Lys	35	25	1.59	0.88–2.88	0.12
Val/Val	Lys/Gln+Gln/Gln	128	83	1.45	0.96–2.16	0.07
Val/Met+Met/Met	Lys/Gln+Gln/Gln	48	37	1.72	1.02–2.89	0.04

OR, odds ratio; CI, confidence interval.

Table 4. Analysis of genes allele in esophageal squamous cell carcinoma carcinogenesis.

	Control allele frequency		Case allele frequency		OR (95% CI)	
Gene	1[a]	2[b]	1[a]	2[b]		*p*-Value
COMT	506	206	275	145	1.30 (1.00–1.68)	0.04
XPD	623	89	350	70	1.40 (0.99–1.97)	0.05

[a]*COMT* (*XPD*) for frequency of presence G (A) allele. [b]*COMT* (*XPD*) for frequency of presence A (C) allele. OR, odds ratio; CI, confidence interval.

Discussion

This is the first study to show that the polymorphisms of *COMT* are associated with the risk of ESCC. These results are important in understanding the role of *COMT* polymorphisms in the pathogenesis of ESCC. There has been considerable interest in a SNP in codon 158 of *COMT*. COMT catalyses the addition of a methyl group to reactive catechol estrogens to convert them into stable methyloxyestrogen conjugates (Creveling & Inoue 1994). Accumulating evidence concerning catechol estrogens suggests that their metabolism may be a contributing factor in tumour formation via direct and indirect genotoxicity (Yager 2000). Therefore, the conversion of catechol estrogens to stable conjugates may be important in preventing ESCC, because metabolites of catechol estrogens have the potential to induce oxidative DNA damage and form DNA adducts. Several studies have examined the relationship between the *COMT* polymorphism and breast cancer risk, ovarian cancer, prostate cancer, renal cell cancer, etc. Three of four peer-reviewed case–control studies (Syvanen et al. 1997, Millikan et al. 1998, Thompson et al. 1998, Dawling et al. 2001) found that the low-activity *COMT* allele was associated with increased breast cancer risk.

Table 2 shows the distribution of the *COMT* Val158Met genotype between controls and ESCC patients. At the genotype level, there were no association of genetic polymorphisms in the *COMT* Vall58Met with risk of ESCC in a population of Yili Prefecture, in Xinjiang, China. Alleles investigated in relation to ESCC carcinogenesis are listed in Table 4. Increased risk of ESCC was suggested in COMT for frequency of presence A allele. Our result indicated that the individual carried the COMT Vall58Met mutation genotype are at increased risk of ESCC.

The *XPD* gene encodes an ATP-dependent DNA helicase involved in NER and in basal transcription as part of the transcription factor TFIIH (Laine et al. 2007). Deficits in repair capacity may lead to genetic instability and carcinogenesis (Berwick & Veneis 2000, Boer 2002). Correlation of its polymorphisms and cancer risk has been studied, but the results remain controversial. A small proportion of the published studies support the conclusion of risk for various types of cancer, such as bladder, breast, melanoma, lung, etc. A meta-analysis (Wang et al. 2008) showed that the *XPD* Gln/Gln genotype was associated with the risk of EC. However, Tables 2 and 4 do not show increased risk of ESCC with the *XPD* Lys751Gln genotype and allele in a population from Yili Prefecture, in Xinjiang, China. This could assist in high-risk screening of humans exposed to environmental carcinogens.

Upon analysis of genotype–genotype interaction in a genetic association, we found that an individual with the combined *COMT* 158 (Val/Met or Met/Met) genotype and *XPD* 751 (Lys/Gln or Gln/Gln) genotype had an increased risk of ESCC, compared with somebody carrying the wild type for both these alleles (Table 3). We presume that an individual who carries the *COMT* Vall58Met mutation genotype has a low catalytic activity and induced DNA damage. Meanwhile, the *XPD* Lys751Gln mutation genotype decreases DNA repair capacity and cannot repair damaged DNA. Therefore, it increases the risk of ESCC carcinogenesis for the individual. Additional studies to confirm these results in other study populations will be beneficial. Furthermore, stratified analysis showed there were no differences in XPD Lys751Gln mutation genotype between ESCC and controls of Han, Uygur and Kazakh ethnicity in Yili Prefecture, in Xinjiang, China (data not shown), but the results were not coincident with the study (Flores-Obando et al. 2010).

In the present study, we are the first to report that an individual carrying the *COMT* Val158Met mutation genotype has an increased risk of ESCC. In addition, the individuals with the combined *COMT* 158 (Val/Met or Met/Met) and *XPD* 751 (Lys/Gln or Gln/Gln) genotypes had an increased ESCC risk. However, these results need to be confirmed with a greater number of patients in different areas

and of different ethnicities, and large, well-designed studies on how *COMT* and *XPD* may influence susceptibility to ESCC are recommended. In addition, the recent genome-wide association study was promising concerning genetic susceptibility.

Declaration of interest

The project was supported by the National Natural Science Foundation (no. 30760223, 30860097) and Xinjiang Key Laboratory of Molecular Biology and Endemic Diseases Grant (no. XJDX 0208-2009-02). The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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