

RESEARCH ARTICLE

MUTYH Association with Esophageal Adenocarcinoma in a Han Chinese Population

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Abstract

Adenocarcinoma of esophagus (AE) is a complex disease, affected by a variety of genetic and environmental factors. Much evidence has shown that the MutY glycosylase homologue (*MUTYH*) plays a key role in the pathogenesis of many cancers. However, there have been no reports on influence on AE in the Han Chinese population. The objective of this study was to investigate this issue. A gene-based association study was conducted using three single nucleotide polymorphisms (SNPs) reported in previous studies. The three SNPs (rs3219463, rs3219472, rs3219489) were genotyped in 207 unrelated AE patients and 249 healthy controls in a case-control study using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). The results revealed that the genotype distribution of rs3219472 differed between the case and control groups (OR=1.66, 95% CI=1.11-2.48, $P=0.012$), indicating that an association may exist between *MUTYH* and AE. These findings support a significant role for *MUTYH* in AE pathogenesis in the Han Chinese population.

Keywords: *MUTYH* - adenocarcinoma of esophagus - SNPs - PCR-RFLP - association study

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Introduction

Adenocarcinoma of esophagus (AE) is the leading cause of death in industrialized countries, and its prevalence is rapidly increasing in China. Many genes are related with the development of AE. DNA base damage is one of the most significant cause of AE, one of the best characterized oxidative DNA lesion is 7,8-dihydro-8-oxoguanine (8-oxo-G), which can give rise to CG→AT transversion mutations, A:8-oxo-G mispairs are proficiently recognized and repaired by *MUTYH* (Markkanen et al., 2013). As reported earlier loss of *MUTYH* function in human cells could lead to accumulation of oxidative damage and genetic instability (Ruggieri et al., 2012). Experimental evidence has confirmed that *MUTYH* plays an important role in many cancers. The *MUTYH* mutation spectrum in Brazilian polyposis patients showed a high detection rate and was used to identify novel pathogenic mutations (Torrezan et al., 2013). In addition 324 Gln/His (rs3219489) *MUTYH* genotypes were found to be associated with an increased colorectal cancer (CRC) risk in Polish patients. Moreover, the decrease efficiency of DNA repair were correlated with the genotypes occurrence in CRC patients (Przybylowska et al., 2013). *MUTYH*-associated polyposis (MAP), caused by biallelic mutations in *MUTYH*, was characterized by

a greatly increased (43% to nearly 100%) lifetime risk of CRC, in the absence of timely surveillance (Brand et al., 2012). Another research suggested that significantly increased cholangiocarcinoma risk was found in individuals with a homozygous variant genotype for rs3219472. It may be a biomarker for screening individuals at high risk of developing the disease in the Han Chinese population (You et al., 2013). In summary, it was found that *MUTYH* had been associated with many diseases in various populations. However, convincing evidence of disease association was found in other populations, other than the Han Chinese. The present study aimed to investigate the association between genetic variations in *MUTYH* and AE in the Han Chinese population.

Materials and Methods

Study subjects

Subjects with Han Chinese ethnicity (n=456) were included in this case-control study. A total of 207 patients with AE, 133 males and 74 females, were recruited from the Department of oncology in the Second Hospital of Shandong University from September 2011 to March 2013. A total of 249 unrelated subjects, 154 males and 95 females, were randomly selected as controls from a health check-up center in the Second Hospital of

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Table 1. Characteristics of Study Population

Index	Case (n=207)	Control (n=249)	p-value
Age(years)	60.15±10.99	59.23±11.21	0.103
Gender(M/F)	133/74	154/95	0.600
Smoker(Yes/No)	178/29	46/203	0.000
Alcohol user(Yes/No)	156/51	57/192	0.000

Table 2. The Distributions of Genotypic and Allelic Frequencies of the Three SNPs in Both Groups

SNPs	Genotype/Allele	Case 207 (%)	Control 249 (%)	p-value	OR-value
rs3219463	CC	75(0.36)	98(0.39)	1	
	CT	103(0.50)	116(0.47)	0.468	0.86 [0.58 1.29]
	TT	29(0.14)	35(0.14)	0.787	0.92 [0.52 1.64]
	C	253(0.61)	312(0.63)		
rs3219472	T	161(0.39)	186(0.37)	0.634	0.94 [0.72 1.23]
	CC	98(0.47)	91(0.37)	1	
	CT	81(0.39)	125(0.50)	0.012	1.66 [1.11 2.48]
	TT	28(0.14)	33(0.13)	0.419	1.27 [0.71 2.26]
rs3219489	C	277(0.67)	307(0.62)		
	T	137(0.33)	191(0.38)	0.099	1.26 [0.96 1.65]
	CC	50(0.24)	65(0.26)	1	
	CG	116(0.56)	139(0.56)	0.719	0.92 [0.59 1.44]
	GG	41(0.20)	45(0.18)	0.554	0.84 [0.48 1.48]
	C	216(0.52)	269(0.54)		
G	198(0.48)	229(0.46)	0.579	0.93 [0.72 1.21]	

Shandong University during the same period. The controls were free of any cancer according to medical history. A structured questionnaire based on interviews and clinical examinations was employed to characterize the subjects and the controls. These included details of medical history, family history of AE and other traditional risk factors (smoking and alcohol drinking) of AE. The clinical and demographic characteristics of the samples are shown in table1. Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Berlin, Germany) (Gu et al., 2012). This study was approved by the Ethics Committee of school of medicine, Shandong University and informed consent was obtained from the participants.

Single-nucleotide polymorphism selection

The single-nucleotide polymorphisms (SNPs) examined here(rs3219472, rs3219489) were the same as previously investigated in the two case-control samples (Przybyłowska et al., 2013; You et al., 2013). The other SNP (rs3219463) is located in the 5'-fanking region and may represent potential functional variant.

Single-nucleotide polymorphism genotyping

At first, the SNPs were genotyped using the PCR-RFLP method. For the PCR-RFLP, the gene sequences harboring the three sites were obtained from Genbank. Primers were designed with primer premier 5.0. The primers and restriction enzymes were: rs3219463, forward, 5'-aagcctttgtaaccacg-3', and reverse, 5'-tcctccgcctgaactag-3', Sbf1; rs3219472, forward, 5'-aagagctacaaggcagaatat-3'. and reverse, 5'-tagttgcttaaacacctcaagt-3', MluCI; rs3219489, forward, 5'-ccccattccagttctcc-3', and reverse, 5'-cagcccgatcaccaggtta-3', BtsI; PCR was carried out in a 20µl reaction volume containing 50ng of genomic DNA ,

10 pmol of each primer, 200 µmmol/L of each dNTP, 4 µl 5 x PCR buffer and 2u taq DNA polymerase. Amplification was carried out with an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 40sec, annealing at a suitable temperature for 40sec, extension at 72°C for 50sec with a final extension at 72°C for 10min. the PCR products containing the SNPs were digested with the suitable restriction enzyme (NEB), and subsequently were separated by electrophoresis on 1.5-2.5% agarose gel. The genotyping accuracy in the samples was confirmed by direct sequencing of the PCR products for certain randomly chosen samples (Cheng et al., 2011).

Statistical analysis

Genotype frequencies of the SNPs detected were tested for Hardy-Weinberg equilibrium. Variations in genotype and allelic frequencies between case and control groups, odds ratios (OR), 95% confidence intervals (CI), and logistic regression analysis were calculated using plink 1.07. Continuous variables were displayed as the mean±standard deviation (SD), and the comparison of continuous variables was carried out using the student's t-test. A p-value <0.05 was considered statistically significant.

Results

Clinical and demographic characteristics of the cases. According to the examination results, the clinical characteristics of the study subjects are shown in Table 1. Age and gender indexes were demonstrated no significant variations but smoking and alcohol drinking between the cases and controls.

Association of the polymorphisms with AE. The distributions of three SNPs were in Hardy-Weinberg equilibrium ($p>0.05$) in both the AE and control groups. The distributions of genotypic and allelic frequencies of these SNPs in each group were shown in Table 2. The genotypes distribution of rs3219472 differed between the case and control groups (OR=1.66,95%CI=1.11-2.48, $P=0.012$), indicating there might be an association between *MUTYH* and AE, on the other hand, the allelic frequencies was no significant difference, after logistic regression analysis were calculated using plink 1.07, removing the effects of traditional factors such as age, gender, alcohol and cigarettes, the genotypes distribution of rs3219472 still have significant differences (OR=1.48,95%CI=1.07-2.45, $P=0.031$) between two groups, which further proves that the onset of AE is associated with the *MUTYH* gene. But the distributions of genotypic and allelic frequencies of the other two SNPs still have no significant difference between the two groups.

Discussion

Adenocarcinoma of esophagus (AE) is a complex disorder resulting from the interaction of a number of genetic and environmental factors. Recent researches have demonstrated that many genes play pivotal roles in the pathogenesis of AE. Numerous studies have shown that damage repair genes play critical role in the pathogenesis

of some cancers in several populations (Miyaiishi et al., 2009; Sliwinski et al., 2009; Picelli et al., 2010; Stanczyk et al., 2011; Santos et al., 2012), indicating the important relationship between the gene *MUTYH* and AE. The main finding of this study is that the same association dose exist in Han Chinese population.

We first investigated the association of *MUTYH* and AE using 207 patients and 249 controls. Significant difference was found in the genotypic frequency distribution of rs3219472 but allelic frequency between the two groups in Han Chinese population studied. After adjusting for age, gender, smoking and drinking alcohol, the genotypic frequency distribution of rs3219472 still has significant difference, but the other two SNPs were not found. Our result demonstrated that the SNPs studied in the *MUTYH* gene are likely to contribute to the AE risk in Han Chinese population.

In conclusion, significant association between SNPrs3219472 and AE were found in our samples. Similar to the role of *MUTYH* gene in cancer of other population, our result suggested that the *MUTYH* gene is likely to be a major susceptibility for AE in Han Chinese population.

Limitation: A number of possibilities may account for the association between the SNPs and AE in this case-control study, including diagnostic heterogeneity, sample size, population stratification and various genetic backgrounds in the different populations. Although not likely, these factors may affect our result.

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