RESEARCH COMMUNICATION

AluYb8 Insertion in the MUTYH Gene and Risk of Early-onset Breast and Gastric Cancers in the Chinese Population

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Abstract

Background: A common polymorphism of the AluYb8 insertion in the MUTYH gene (AluYb8MUTYH), which led to the increase of oxidative DNA damage and acceleration of chronic diseases, was previously detected. Considering the relationship between carcinogenesis and oxidative stress, an investigation was held on whether the common variant of the MUTYH gene increases the risk for gastric and breast cancers. Methods: The AluYb8MUTYH allele frequencies of 545 breast cancer patients and 762 gastric cancer patients were analyzed and compared with that of the healthy control group using the Chi-square test. The binary logistic regression model was used to examine the association between the polymorphism genotypes and cancer risk. Genomic DNA specimens from the investigated population were tested by polymerase chain reaction in agarose gel electrophoresis. According to the insertion absence or presence of the variant segment, the patterns for the AluYb8MUTYH genotypes were classified as a homozygous of absence/absence (A/A) and presence/presence (P/P) or a heterozygous of absence/presence (A/P). Results: The variant allele frequency (insertion present, P) was inclined to be enhanced in breast cancer patients as compared with the normal female controls (46.8% versus 43.3%), and also, in gastric cancer patients, as compared with the general normal controls (45.1% versus 43.9%). However, a significantly different P allele frequency was only detected between the early-onset breast cancer patients (<55 years old) and their counterpart female controls (46.6% versus 40.9%, p=0.042; OR=1.26, 95% CI, 1.01–1.56), as well as between the early-onset gastric cancer patients and their respective controls (49.2% versus 41.3%, p=0.042; OR, 1.37; 95% CI, 1.02–1.85). Comparisons on the genotypes of AluYb8MUTYH show that this variation of MUTYH has also a significantly higher prevalence in the early-onset cancer patients, either in breast or gastric cancer patients, than that in their counterpart controls. Conclusions: The AluYb8MUTYH allele frequency can be associated with the early-onset breast and gastric cancer in the Chinese population. Probably, there is importance in screening the carriers with the susceptibility alleles to evaluate their risk of breast and gastric cancer for further research.

Keywords: MUTYH gene - Alu insertion - breast cancer - gastric cancer

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Introduction

Oxidative stress is defined as the excessive production of reactive oxygen species in the presence of diminished antioxidant substances. The present findings provided a potential mechanism, in which oxidative stress induced DNA damage playing a role in the pathogenesis of various cancers (Loft and Poulsen, 1997; Bartsch and Nair, 2006). Increased oxidative stress is widely known to harm all cellular macromolecules, and DNA is the most important biological target since it has a limited chemical stability. The most frequently reported oxidative DNA damage is 8-hydroxy-2'deoxyguanosine (8-OHdG), and an increased 8-OHdG level has been demonstrated in cancer patients (Niedernhofer et al., 2006; Valavanidis et al., 2009).

Fortunately, the harmful consequences of DNA damage can be prevented by the efficient repair mechanisms in cells (Robertson et al., 2009). Oxidized DNA bases are substrates for several overlapping repair pathways, mainly the base excision repair (BER) system (Zharkov 2008). Several DNA glycosylases are associated with the BER system. Human MutY glycosylase homolog (MUTYH) is specifically involved in the removal of adenines mismatched with 8-OHdG resulting from DNA replication errors and DNA recombination (Michaels et al., 1992). Cooperating with 8-oxoG glycosylase (hOGG1) and human MutT homolog, the MUTYH protein can protect the cell from the mutagenic effects of 8-OHdG

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(Ohtsubo et al., 2000). On the other hand, the ability of repairing damaged DNA can significantly decrease the gene mutation in the BER system (Shinmura et al., 2001). Alu sequences are short interspersed elements, which account for more than 10% of the human genome (Lander et al., 2001). The Alu element is the most abundant family of repetitive DNA sequences in the human genome, and is frequently present in noncoding regions, such as introns, 3' untranslated regions of genes, and intergenic regions (Batzer and Deininger, 2002). As the youngest subfamily, some of the AluY show in a polymorphism (Dagan et al., 2004). More studies indicate that Alu insertion in the human genome is associated with diseases and AluYb8 is one of the most active in the Alu subfamily (Deininger and Batzer, 1999; Carroll et al., 2001).

Previously, our group has identified a novel MUTYH polymorphism, AluYb8MUTYH (AluYb8 insertion in intron 15 of the MUTYH gene), in the Chinese population. The homozygous genotype of this variation (the insertion present in homozygous, P/P) showed an age-related distribution and was associated with the increased levels of 8-OHdG and IL-1 among the Chinese "healthy" population. This suggests that the P/P genotype of the AluYb8MUTYH variant may be a candidate modifier for the common age-related diseases (Sun et al., 2010).

Cancer is one of the major chronic age-related diseases (Federico et al., 2007; Khansari et al., 2009). The worldwide epidemic of cancers has presented a major public heath problem. Currently, about 2 million people die from cancer in China each year (Duan et al., 2009). Moreover, the germline mutations of MUTYH, which can lead to autosomal recessive colorectal adenomatous polyposis and cancers, were discovered recently (Al-Tassan et al., 2002; Jenkins et al., 2006). Regarding the roles of oxidative DNA damage in cancer, the novel MUTYH gene polymorphism is hypothesized to be a risk factor for common cancers. Gastric and breast cancers are the third and sixth most common cancers, respectively, in the Chinese population (Duan et al., 2009). In this paper, our hypothesis is tested using a case-control study of the common variant AluYb8MUTYH in both gastric and breast cancer patients.

Materials and Methods

Subjects

A. Breast cancer cases: A total of 545 unrelated female breast cancer patients, who had been diagnosed by histopathology in the hospitals, were randomly recruited in Nanjing, Jiangsu province in the eastern part of China from October 2008 to December 2009. In China, the median age of the onset of breast cancer is around 60 years old. Fifty five years old (5 years younger compared with the median age) was chosen as the age limit to divide the breast cancer patients into two subgroups, the patients diagnosed at the age <55 years old (the early-onset patients) and the patients ≥55 years old (the late-onset patients). Among the breast cancer patients recruited in this study, 337 were diagnosed at the age of <55 years old and 208 at the age ≥55 years old.

B. Gastric cancer cases: A total of 762 unrelated gastric cancer patients, who had been diagnosed by histopathology, were randomly recruited in the eastern part of China, Lujiang in the Anhui province and Nanjing in the Jiangsu province, from October 2008 to December 2009. In China, the median age of the onset of gastric cancer is also around 60 years old. Fifty five years old was chosen as the age limit to divide the gastric cancer patients into two groups as mentioned above in breast cancer patients. From a total of 762 gastric cancer patients, 178 were in the early-onset (<55 years old) group and 584 were in the late-onset (≥55 years old) group.

C. Healthy controls: Five hundred and forty five healthy controls, matched with the breast cancer patients for each case by age, were selected from the female subjects who came to the hospital for a routine physical examination from October 2008 to December 2009. Individuals suffering from cancer had been excluded from the healthy controls based on the clinical and laboratory test results.

Seven hundred sixty two healthy Chinese individuals, matched with the gastric cancer patients for each case by sex and age, were selected from people who came to the hospital for a routine physical examination from October 2008 to December 2009. Individuals suffering from cancer had been excluded from the controls based on the clinical and laboratory test results. Informed consent was obtained from every patient and control subject, and the study protocol was approved by the local authorities at Nanjing University School of Medicine.

DNA extraction

Blood samples from all the subjects were collected, placed in EDTA solution, and stored at -70 °C. Genomic DNA was extracted from the peripheral lymphocytes using a Genomic DNA Extraction kit (TIANGEN Biotech Co. Ltd., Beijing, China) according to the manufacturer's instructions.

Variation screening in MUTYH gene

The primers were designed to amplify the sequence of intron15, including the polymorphism of the AluYb8 insertion, in the MUTYH gene:

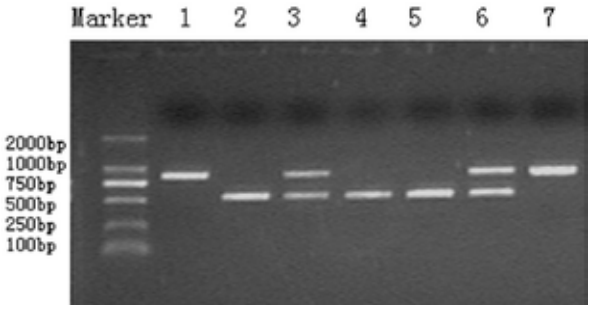

Figure 1. Agarose gel electrophoresis of the PCR products covering partial MUTYH intron 15. The genomic amplification products were from 7 cases in China. Case 2, 4, and 5 presented the A/A products, which have a 536 bp fragment. Case 3 and 6 presented A/P products, which have 536 and 880 bp fragments, respectively. Case 1 and 7 presented P/P products, which have a 880 bp fragment.

The forward, 5'-ATTGTGGGGTGCAGGGTAG-3';
The reverse, 5'-GGAAAGAACATCGCTACGG-3'.
Genomic DNA specimens from the investigated population were tested by polymerase chain reaction (PCR) in agarose gel electrophoresis. The PCR products were separated in 1% agarose gels (Invitrogen, Carlsbad, CA) to assess the variation pattern in the MUTYH gene. According to the presence or absence of AluYb8MUTYH, the MUTYH genotypes were classified as homozygote without AluYb8 insertion (absence/absence, A/A), homozygote with AluYb8 insertion (presence/presence, P/P), and heterozygote (absence/presence, A/P). The product with only the A/A genotype showed a 536 bp fragment while the P/P genotype showed a 880 bp fragment. However, the heterozygous A/P had both the 536 and 880 bp fragments (Figure 1).

Statistical analysis

All statistical analyses were carried out using the statistical program SPSS version 11.5 (SPSS for Windows, Rel. 11.5.0. 2002. Chicago: SPSS Inc., http://www.spss.com/). Comparisons of the allelic frequencies for cancer patients and normal controls were performed using the Chi-square test (Sun et al., 2010).

The binary logistic regression model was used to examine the association between genotypes of the polymorphism and cancer risk. The odds ratio (OR) was shown with 95% confidence intervals (CI). All tests were 2-sided, and a p value of less than 0.05 was considered statistically significant.

Results

Association of AluYb8MUTYH with the risk of breast cancer

The P allele frequency increased more in breast cancer patients than that in the healthy female controls (46.8% versus 43.3%, P=0.11; OR, 1.15; 95% CI, 0.97–1.36), but no significant difference was found. However, early-onset breast cancer patients showed a significantly higher P allele frequency as compared with their age counterpart female controls (46.6% versus 40.9%, p=0.042; OR=1.26; 95% CI, 1.01–1.56) (Table 1). Pairwise comparisons also showed that individuals with the P allele (including A/P heterozygous and P/P homozygous genotypes) have a significantly higher prevalence of breast cancer than individuals with the A/A genotype. This indicates a dominant model for polymorphism of AluYb8MUTYH during the early age (<55 years) (P=0.014; OR=1.51; 95% CI, 1.09–2.08).

Association of the AluYb8MUTYH with the risk of gastric cancer

The P allele frequency was greater in gastric cancer patients than that in the normal controls (45.1% versus 43.9%, P=0.54; OR, 1.05; 95% CI, 0.91–1.21), but no significant difference was found. Statistical analysis showed that the early-onset gastric cancer patients had a significantly higher P allele frequency as compared with the healthy controls (49.2% versus 41.3%, p=0.042; OR=1.37; 95% CI, 1.02–1.85) (Table 2). Pairwise

Table 1. Association of the AluYb8MUTYH with the Risk of Breast Cancer by Age at Diagnosis

Ages	Allele/Genotype		BC patients	Controls	P-value	OR	(95% CI)
<55 years old	Alleles	A	360 (53.4%)	398 (59.1%)	0.042	1.26	(1.01-1.56)
		P	314 (46.6%)	276 (40.9%)			
	Genotypes in	A/A+A/P	266 (78.9%)	274 (81.3%)	0.44	1.16	(0.80-1.70)
	Recessive Model	P/P	71 (21.1%)	63 (18.7%)			
	Genotypes in	A/A	94 (27.9%)	124 (36.8%)	0.014	1.51	(1.09-2.08)
	Dominant Model	A/P+P/P	243 (72.1%)	213 (63.2%)			
≥55 years old	Alleles	A	220 (52.9%)	220 (52.9%)	1	1	(0.76-1.31)
		P	196 (47.1%)	196 (47.1%)			
	Genotypes in	A/A+A/P	160 (76.9%)	165 (79.4%)	0.56	1.15	(0.72-1.83)
	Recessive Model	P/P	48 (23.1%)	43 (20.6%)			
	Genotypes in	A/A	60 (28.9%)	55 (26.4%)	0.58	0.89	(0.58-1.36)
	Dominant Model	A/P+P/P	148 (71.1%)	153 (73.6%)			

A, absence of insertion; P, presence of insertion; BC, breast cancer

Table 2. Association of the AluYb8MUTYH with the Risk of Gastric Cancer by Age at Diagnosis

Ages	Allele/Genotype		GC patients	Controls	P-value	OR	(95% CI)
<55 years old	Alleles	A	181 (50.8%)	209 (58.7%)	0.042	1.37	(1.02-1.85)
		P	175 (49.2%)	147 (41.3%)			
	Genotypes in	A/A+A/P	126 (70.8%)	145 (81.5%)	0.019	1.81	(1.10-2.98)
	Recessive Model	P/P	52 (29.2%)	33 (18.5%)			
	Genotypes in	A/A	55 (30.9%)	64 (36.0%)	0.31	1.26	(0.81-1.96)
	Dominant Model	A/P+P/P	123 (69.1%)	114 (64.0%)			
≥55 years old	Alleles	A	656 (56.2%)	646 (55.3%)	0.71	0.97	(0.82-1.14)
		P	512 (43.8%)	522 (44.7%)			
	Genotypes in	A/A+A/P	464 (79.5%)	453 (77.6%)	0.43	0.89	(0.68-1.18)
	Recessive Model	P/P	120 (20.5%)	131 (22.4%)			
	Genotypes in	A/A	192 (32.9%)	193 (33.0%)	0.94	1.01	(0.79-1.29)
	Dominant Model	A/P+P/P	392 (67.1%)	391 (67.0%)			

A, absence of insertion; P, presence of insertion; GC, gastric cancer

comparisons also showed that the P/P genotype group have a significantly higher prevalence in early-onset patients than that in the healthy controls (29.2% versus 18.5%, P=0.019; OR=1.81; 95% CI, 1.10–2.98). This probably indicates a recessive model for the polymorphism of AluYb8MUTYH in the early-onset gastric cancer.

Discussion

Previous studies about MUTYH mutations primarily focused on the relationship between single nucleotide variants, especially G396D and Y179C, and colorectal cancer (Al-Tassan et al., 2002; Jenkins et al., 2006; Cleary et al., 2009; Theodoratou et al., 2010). Al-Tassan et al. (2002) reported that compound heterozygotes for the nonconservative missense variants, Y179C and G396D, significantly increase the risk for colorectal adenoma or carcinoma (OR about 50). Theodoratou et al. (2010) performed a large-scale meta-analysis to refine the colorectal cancer risk estimates associated with the MUTYH variants G396D and Y179C. Their reports state that bi-allelic carriers for the MM genotype of the combined MUTYH defects, G396D and Y179C/G396D compound heterozygotes, were associated with the significant increase in the colorectal cancer risk (OR = 28.3, 23.1 and 21.6, respectively). Previous studies on the relationship between MUTYH mutations and the risk of breast or gastric cancer were rare (Beiner et al., 2009; Vogt et al., 2009; Wasielewski et al., 2010). Wasielewski et al. (2010) suggested that heterozygous MUTYH mutations are associated with a breast cancer phenotype, even though there was incomplete co-segregation of MUTYH mutations with breast cancer. Vogt et al. (2009) reported that the relative risks for several extraintestinal malignancies increased in patients with MAP.

AluYb8MUTYH was a 326-bp insertion and located at 479 bp downstream of the MUTYH gene exon 15 (IVS15+479ins326). In a separate study, Out et al. (2009) reported that an Alu polymorphism affected the data sequencing of their test. Of the 88 samples, 48 were heterozygous and 22 were homozygous for the insertion, yielding an allele frequency of 52%. However, there were no further studies on Alu insertion. In this study, the AluYb8MUTYH allele frequency increased in cancer patients, but a statistically significant increase of the frequencies was only detected in the relative young patients during the onset of cancer, either breast or gastric cancer, at <55 years old. This suggests that this AluYb8MUTYH variant was a risk factor for breast and gastric cancers, especially in the early ages. Although a statistically significant difference was achieved in the above analysis, the "p values" were at the borderline of the limited sample size of early-onset cancer patients. Thus, for further study, the sample size must be expanded. When the frequencies of genotypes between cancer patients and healthy controls were compared, a dominant model was found for breast cancer and a recessive model was found for gastric cancer. This may be related with the different characteristics in carcinogenesis between breast and gastric cancers. Thus, further studies must focus on AluYb8MUTYH and the tumor pathogenesis.

In our previous study, the AluYb8 retrotransposon inserted in the MUTYH gene was demonstrated to be hypermethylated in genomic DNA with MSP (Sun et al., 2010). The hypermethylation status and high GC content of the Alu sequence have been reported to affect the dynamics of the surrounding nucleotide sequence, such as the cis-regulatory element, while many large introns of eukaryotes cover cis-regulatory elements, including enhancers or silencers (Halder et al., 1995; Dirksen et al., 2003). Since intron 15 is the largest intron in the MUTYH gene, consisting of 1078 bp, exploring the possible effect of the AluYb8 inserted on gene regulation is an attractive option. By providing alternative splice sites in pre-mRNAs, Alu RNAs embedded into the introns were shown to be important mediators of alternative splicing (Kreahling and Graveley, 2004; Hasler and Strub, 2006). The Alu consensus sequence contains 9 potential 5' splice sites and 14 potential 3' splice sites (Makalowski et al., 1994; Sorek et al., 2002), most of them being present on the minus strand. Pathologies such as the Alport and the Sly syndromes are known to be caused by mutations that result in constitutive inclusion of an Alu exon (Knebelmann et al., 1995; Vervoort et al., 1998). More recently, the alternative inclusion of an Alu exon was discovered to lead to a genetic disease. A mutation in intron 6 of the CTDP1 gene, creating an alternatively spliced Alu exon, results in CCFDN (congenital cataracts, facial dysmorphism, and neuropathy) syndrome (Varon et al., 2003). The relationship between AluYb8MUTYH and mRNA expression must be explored in further studies.

The identified AluYb8MUTYH was associated with increased DNA oxidation and age-related diseases in the Chinese population (Sun et al., 2010). The 8-OHdG concentration of blood cell DNA was also associated with the genotypes of AluYb8MUTYH (P<0.001). Those results suggested that the MUTYH gene variation was needed for the accumulation of DNA oxidative damage in the cells. In this study, the early-onset gastric cancer and breast cancer patients showed a significantly higher P allele frequency as compared with their counterpart controls. Our data suggested that the P allele of MUTYH contribute significantly to age-related diseases by the accumulation of 8-OHdG in genomic DNA. This can be the basic mechanism since the AluYb8MUTYH variant was a risk factor for two kinds of common cancers, gastric and breast cancers. The association between AluYb8MUTYH and other common cancers needs further study.

In conclusion, the AluYb8MUTYH allele frequency was associated with the early-onset of gastric and breast cancer in the Chinese population. Probably, there is importance in screening the carriers with the susceptibility alleles to evaluate their risk of breast and gastric cancer for further research.

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