

# Association of the p73 Gene G4C14-to-A4T14 Polymorphism with Increased Gastric Cancer Risk in a Northwestern Chinese Population

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**OBJECTIVE** To evaluate the p73 gene G4C14-to-A4T14 double nucleotide polymorphism with both increased gastric cancer (GC) risk and different histological subtypes of GC in a northwestern Chinese population.

**METHODS** Genotyping of the polymorphism of the p73 gene was conducted with PCR-CTPP.

**RESULTS** All 385 GC patients including 305 diffuse-type and 80 intestinal-type cases and 412 healthy controls were investigated. The frequencies of p73 AT/AT, AT/GC, and GC/GC genotypes were 28.1%, 47.1%, and 24.8% in the controls, and were 22.0%, 45.0%, and 33.0% in GC cases respectively; the GC/GC homozygote frequency was higher in GC cases, mainly in diffuse type compared to the controls with OR = 1.71 (1.16~2.51) and 1.87 (95% CI, 1.24~2.81) respectively. The results showed that carriers of the p73 G4A GC/GC homozygote had a 1.71-time higher risk of GC, especially of the diffuse-type GC compared to the controls. The carriers of the AT/GC heterozygote also had a slightly increased risk of GC cancer, mainly on intestinal-type GC. This is the first report that the p73 G4A double-nucleotide polymorphism is associated with an increased risk of diffuse-type gastric cancer.

**CONCLUSION** The p73 G4A GC/GC genotype is associated with an increased risk of gastric cancer, especially of the GC diffuse-type.

**KEY WORDS:** p73 gene, polymorphism, gastric neoplasm, pathologic subtype.

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## Introduction

Gastric cancer (GC) is the second most common malignant disease worldwide, and the first in the northwestern part of China<sup>[1]</sup>. The pathogenesis of GC and the molecular genetic events that contribute to development of the disease are still poorly understood. The p73 gene has been mapped on the 1p36.33 region<sup>[2-4]</sup>, with remarkable sequence identity to the DNA-binding, transactivation and oligomerization domains of p53. It has 2 natural-linked polymorphisms in exon 2 consisting of a double-nucleotide substitution G-A and C-T at positions 4 and 14 (G4C14 to A4T14, termed G4A hereafter), which exist in a region of the transcript that could form a stem-loop structure and affect gene expression<sup>[5]</sup>. Therefore this substitution could have functional consequences. Some reports have shown that the p73 G4A polymorphism was associated with cancers of the cervix, endometrium, head and neck, esophagus, colon and lung<sup>[6-12]</sup>. There are only a few reports on the association between the p73 G4A polymorphism and GC risk. Hamajima et al.<sup>[13]</sup> reported that p73 G4A double nucleotide polymorphisms (DNP) were not related to GC and other digestive tract cancers in Japanese; and Ge et al.<sup>[14]</sup> indicated in their study of Chinese people living in Hebei, China, that the p73 G4A

GC/AT genotype increased the risk of gastric cardiac adenocarcinoma in those without a family history of upper gastrointestinal cancer. To our best knowledge, there has been no study on the relationship between the p73 G4A polymorphism and different histological subtypes of GC.

This work was aimed to evaluate the genetic associations of the p73 gene G4A polymorphism with both increased GC risk and different histological subtypes of GC in a northwestern Chinese population.

## Materials and Methods

### Study subjects

This study investigated GC cases and healthy controls in Wuwei city, Gansu Province in northwestern China where the GC incidence (209.8 per 100,000 people) ranked first in China<sup>[1]</sup>. Samples of GC cases were surgically resected specimens which were separated into cancer tissues and para-cancer normal tissues (PCNT) separately, and were obtained from 4 hospitals in the city. All of the GC cases were diagnosed histopathologically, and none of the patients had received any other treatment such as chemotherapy before operation. The controls were cancer-free healthy volunteers born in the same district matched with age, gender and ethnic background by checking examinee records in these hospitals. All of these samples were stored at  $-70^{\circ}\text{C}$ .

### Classification of GC histological subtypes

The GC histological subtypes were classified into intestinal-type and diffuse-type GC as defined by Lauren<sup>[15]</sup>.

### DNA extraction

Genomic DNA of the controls was extracted from EDTA anticoagulated peripheral blood according to a standard proteinase K digestion and phenol/chloroform extraction method. DNA of tissues from GC cases was separately extracted from homogenized GC tissues and PCNT specimens by the same method.

### Genotyping of the p73 G4A polymorphism (PCR-CTPP)

The genotyping was performed using the polymerase chain reaction with the confronting two-pair primer (PCR-CTPP) method<sup>[8,9,13,16]</sup>. Each PCR reaction mixture (25  $\mu\text{l}$ ) contained 0.2  $\mu\text{mol}$  of each primer, 1.0 mM  $\text{MgCl}_2$ , 200  $\mu\text{M}$  of each dNTP, 1.6 U *Taq* DNA polymerase and 20 ng of DNA template. Primers were F1: 5'-TTA GCC CAG CGA AGG TGG-3', R1: 5'-CCT TCC TTC CTG CAG AGC G-3', F2: 5'-GGC CTC CAA GGG CAG CTT-3' and R2: 5'-CCA CGG ATG GGT CTG ATC C-3'.

PCR conditions were as follows: initial denaturing ( $95^{\circ}\text{C}$  for 5 min), followed by 30 cycles of denaturing ( $95^{\circ}\text{C}$  for 1 min), annealing ( $62^{\circ}\text{C}$ , 1 min) and extension ( $72^{\circ}\text{C}$ , 1 min); and a final extension ( $72^{\circ}\text{C}$ , 5 min). The

PCR products were analyzed by 2% agarose gel electrophoresis. Alleles were coded as follows: GC/GC = 428 bp + 194 bp, GC/AT = 428 + 270 + 194 bp, and AT/AT = 428 bp + 270 bp<sup>[8]</sup>.

### Statistical analysis

Hardy-Weinberg equilibrium of alleles at individual loci was assessed by  $\chi^2$  statistics. Genotype frequencies were analyzed by the odds ratio (OR) and 95% confidence interval (95% CI). Differences in age and sex between the 2 groups and differences of genotype frequencies in different histological subtypes were assessed by the  $\chi^2$  test. The statistical analysis was performed with SPSS 10.0 software. A value of  $P < 0.05$  was regarded as significant.

## Results

We investigated 385 GC cases and 412 healthy controls between October, 2002 and March, 2005. There were 308 (80%) males and 77 (20%) females in the GC group; the average age was 54.7 years (36–70 years). The controls were matched with age and gender by checking examinee records in these hospitals (Table 1).

**Table 1. Common characteristics of the studied GC cases and the controls (n, %).**

	GC n = 385	Controls n = 412	$\chi^2$	P
Age, years			0.17	> 0.50
$\leq 50$	127 (33.0)	130 (31.5)		
$> 50$	258 (67.0)	282 (68.4)		
Sex			0.04	> 0.75
Male	308 (80.0)	332 (80.6)		
Female	77 (20.0)	80 (19.4)		

There were 305 (79.2%) diffuse-type cases and 80 (20.8%) intestinal-type cases in the GC group. The p73 G4A polymorphism of the 385 GC cases and 412 controls were evaluated in the study.

p73 G4A alleles could not be detected in 12 (3.2%) of the 385 GC samples (in both GC tissue and PCNT), so they were evaluated in the remaining 373 GC patients including 295 (79.1%) diffuse-type cases and 78 (20.9%) intestinal-type cases. All genotype frequencies were in Hardy-Weinberg equilibrium, and there were no statistical differences in genotype frequencies between cancer tissues and para-cancer normal tissues in the cases group (data not shown).

The frequencies of the p73 AT/AT, AT/GC, and GC/GC genotypes were 28.1%, 47.1%, and 24.8% in the controls, and 22.0%, 45.0%, and 33.0% respectively in GC cases. Using the AT/AT as the referent, the GC/GC homozygote frequency was higher in GC cases, mainly in diffuse type GC cases compared to the controls, with OR = 1.71 (95% CI, 1.16–2.51) and 1.87 (1.24–2.81)

**Table 2. p73 gene G4A genotype frequencies in controls, GC cases and in different histological subtypes of GC in a studied Chinese population (n, %).**

Locus	p73 G4A		
	AT/AT	AT/GC	GC/GC
Controls (n = 412)	116 (28.1)	194 (47.1)	102 (24.8)
GC cases (n = 373)	82 (22.0)	168 (45.0)	123 (33.0)
OR (95% CI)	1.00	1.23(0.86~1.74)	1.71(1.16~2.51)
Diffuse-type GC (n = 295)	64 (21.7)	126 (42.7)	105 (35.6)
OR (95% CI)	1.00	1.18 (0.81~1.72)	1.87 (1.24~2.81)
Intestinal type GC (n = 78)	18 (23.1)	42 (53.8)	18 (23.1)
OR (95% CI)	1.00	1.40 (0.70~2.54)	1.14 (0.56~2.30)

respectively. The AT/GC heterozygote frequency was higher in intestinal GC cases compared to the controls, with OR = 1.40 (95% CI, 0.70~2.54) (Table 2).

## Discussion

It has been reported that transient over-expression of p73 induced G1 cell cycle arrest and apoptosis in a p53-like manner, and activated the transcription of p53-responsive genes, such as p21Waf1<sup>[17,18]</sup>, suggesting that p73 has tumor-suppressor functions. Otherwise, the p73 gene has some significant differences from p53: *i*) until now, there is no conclusive evidence showing that p73 is inactivated in malignant diseases<sup>[19,20]</sup>; for example, a p73 mutation is very rare in human cancers including those with a loss of heterozygosity (LOH); even the original cloning report noted that the remaining copy of the p73 gene in neuroblastoma cells was the wild type<sup>[20]</sup>; *ii*) wild p73 is expressed at high levels in some cancers such as lung, prostate, stomach and kidney, but at low levels in all normal tissues<sup>[7,19,21-32]</sup>; *iii*) unlike p53 -/- mice, p73 knockout mice do not show increased rates of spontaneous tumorigenesis<sup>[33]</sup>; *iv*) p73 expression is not induced by UV irradiation or actinomycin D, which can induce p53 and differently regulate cellular p53-target genes<sup>[34,35]</sup>; and *v*) p73 inactivation is not required for virus-induced tumor development, and none of the p53-inactivating viral oncoproteins, such as adenovirus E1B 55K, SV40 T antigen, and human papillomavirus E6, can destabilize p73<sup>[35,36]</sup>. These findings suggest that p73 may augment, rather than inhibit tumor development. So, it is still not certain whether p73 is a tumor suppressor or an oncogene.

The results from the present study showed that carriers of the p73 G4A GC/GC homozygote had a 1.71-time higher risk of GC, especially of diffuse-type GC compared to the controls. The carriers of an AT/GC heterozygote also had a slightly increased risk of GC cancer, mainly of the GC-intestinal type. This is the first report that the p73 G4A double-nucleotide polymorphism is associated with an increased risk of diffuse-type gastric cancer.

In our study, p73 G4A alleles were not detected in 12 (3.12%) of the 385 GC cases, including 10 (3.28%)

diffuse-type and 2 (2.50%) intestinal -type GC cases. Because p53 and some other genes could be amplified in all of these samples<sup>[37]</sup>, it is suggested that LOH occurred on chromosome 1p36.33 of these GC cases. It has been reported that the p73 gene exhibits frequent LOH in a variety of human cancers such as those of the prostate, lung, esophagus, breast, ovary and colon, as well as neuroblastoma and colorectal adenoma<sup>[6,30,32,38-41]</sup>. Yokozaki et al.<sup>[40]</sup> found LOH of p73 in 37.5% (12/32) of Japanese GC cases, but found no p73 mutations. Cai et al.<sup>[3]</sup> reported that p73 LOH was detected in 64% (9/14) of esophageal carcinoma cases. Tannapfel et al.<sup>[41]</sup> analyzed 68 GC cases and brought forward the view that p73 mutation was rare in GC tissues. The frequency of LOH in our present study was much lower than those of above reports, suggesting that LOH of the p73 gene is not significantly related to GC risk in the population studied.

Our results showed that diffuse-type cancer was dominant in the GC cases. This differs from the common rule that intestinal-type GC is dominant in the high-incidence GC region, while diffuse-type GC is more common in the low-incidence region; for example, intestinal-type GC is dominant in Japan where GC has a high incidence, whereas diffuse-type GC is common in Western countries where the GC incidence is very low<sup>[35,36]</sup>.

In conclusion, p73 G4A GC/GC homozygotes are associated with an increased diffuse-type GC risk in our northwestern Chinese population. The study also reveals that diffuse-type GC was dominant (79.2%) in the highest GC-incidence area, differing from the common rule.

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