• GASTRIC CANCER •

Association of tumor necrosis factor genetic polymorphism with chronic atrophic gastritis and gastric adenocarcinoma in Chinese Han population

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Abstract

AIM: To investigate the association of TNF polymorphisms with chronic atrophic gastritis (CAG) and gastric adenocarcinoma in Chinese Han patients.

METHODS: The TNFa-e 5 microsatellites and 3 RFLP sites were typed using PCR technique, followed by high-voltage denaturing PAGE with silver staining and restriction enzyme digestion respectively in specimens from 53 patients with CAG and 56 patients with gastric adenocarcinoma and 164 healthy controls. The PCR products were cloned and sequenced.

RESULTS: The frequency of TNF-β Ncol*1/2 genotype was higher in patients with chronic atrophic gastritis than in healthy controls, but no significant difference was observed (60.38% *vs* 46.34%, *P*=0.076). The frequency of TNa10 allele was significantly higher in patients with chronic atrophic gastritis than in healthy controls (19.81% *vs* 11.89%, *P*=0.04). However, it did not relate to age, gender, atrophic degree or intestinal metaplasia in patients with chronic atrophic gastritis. The frequency of TNF-β Ncol*1/2 and d2/d6 genotypes were significantly higher in patients with gastric adenocarcinoma than in healthy individuals(*P*>0.05). However, TNF-β Ncol*1/2 and d2/d6 genotypes did not relate to age, gender, grade of differentiation and clinicopathologic stage in patients with gastric adenocarcinoma. The frequency of TNFa6b5c1 haplotype homozygote was significantly lower in patients with gastric adenocarcinoma than in healthy controls (1.79% *vs* 15.85%, *P*=0.006).

CONCLUSION: TNFa10 allele may be a risk factor for chronic atrophic gastritis. TNF-β Ncol*1/2 and d2/d6 genotypes are associated with the susceptibility to gastric adenocarcinoma, whereas TNFa6b5c1 haplotype homozygote may contribute to the resistance against gastric adenocarcinoma.

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INTRODUCTION

Gastric adenocarcinoma is one of the most frequent malignant diseases in the world, but the causes of gastric cancer remains unclear. The crude mortality rate of stomach cancer in China was 25.2 per 10^5 (32.8 per 10^5 for males and 17.0 per 10^5 for females), which comprised 23.2% of the total death of cancer from 1990 to 1992, making stomach cancer the leading cause of the death among cancers[1].

Tumor necrosis factor (TNF) is a multifunctional cytokine and its anti-tumor effect has attracted particular attention. TNF was found at a higher concentration in patients with malignant tumor. Forones *et al.*[2] reported increased TNF-α expression in the sera of patients with advanced gastric cancer. TNF-α mRNA was markedly increased in gastric carcinoma tissue[3]. Recombinant TNF has been demonstrated to have considerable treatment effect on gastric cancer *in vitro*[4,5].

The gene for TNF-α and lymphotoxin-α (TNF-β), referred to as the TNF locus, are tandemly arranged within a 7-kilobase region in the HLA on the short arm of chromosome 6. HLA has recently been found to contribute to cancer development[6-9]. Several RFLP sites and 5 microsatellites within the TNF gene have been identified. Some studies have shown that TNF individual alleles were correlated to secretion from activated monocytes[10-13]. Furthermore, some experiments found that TNF secretion was also associated with TNF haplotypes, not only individual alleles[14]. These findings suggest that TNF polymorphisms may play a role in the pathogenesis of several autoimmune, infectious and neoplastic diseases.

Chronic atrophic gastritis (CAG) is believed to be the precancerous lesion of gastric adenocarcinoma. In the present study, we examined whether TNF genetic polymorphisms were associated with CAG and gastric adenocarcinoma in Chinese Han population. Additionally, we determined whether the associations between TNF genetic polymorphisms and CAG and gastric adenocarcinoma varied with clinicopathologic features of the 2 diseases.

MATERIALS AND METHODS

Patients and genomic DNA extraction

The subjects of this study included 56 patients with gastric adenocarcinoma (43 males, 13 females; mean age 55.6±12.2 years), 53 patients with CAG (32 males, 21 females; mean age 53.5±11.4 years), and 164 unrelated healthy individuals (113 males, 51 females; mean age 52.5±11.3 years) from Chinese Han population in Hubei province of China. The diagnoses of gastric adenocarcinoma and CAG were confirmed by histopathology examinations. Genomic DNA was extracted

from venous blood by a salting out procedure with minor modifications.

TNF polymorphism typing

Five microsatellites were amplified using a single step PCR reaction with primers described by Pociot *et al.*[15]. TNF microsatellite alleles were typed by a 60 g/L polyacrylamide, 0.4-mm sequencing gel, followed by silver nitrate staining. Fragments were sized using DNA markers and simultaneously typed with known alleles derived from the cloned PGEM-T vector.

The sites in the first intron of TNF-β marked by AspH1 and *Nco*1 were analyzed using PCR and endonuclease digestion. BSIHKA1, an isoschizomer of AspH1 (New England Biolabs, Beverley, MA) was used instead of AspH1. The digested products were electrophoresed in 1 g/L ethidium bromide-stained 15 g/L agarose gels. Similarly, the TNF-308 polymorphism was examined by PCR amplification and *Nco1* digestion. The digested products were separated on a 100 g/L non-denaturing polyacrylamide gel. Alleles were visualized as described for TNF microsatellite typing.

Cloning and sequencing

The PCR products of 5 microsatellites were purified and ligated with PGEM-T vector. High efficiency JM 109 competent cells were used in the process of transformation. Needed transformants were obtained by blue/white color screening and standard ampicillin selection. Recombinant plasmid DNA were isolated and identified. Sequencing was done on ABI 377 DNA sequencer.

Statistical analysis

Allele frequencies in patients and control groups were calculated by direct counting. The Chi-square test and Fisher's exact test were used for statistical analysis. Hardy-Weiberg equilibrium was tested in a $2 \times n$ analysis using Chi-Square.

RESULTS

The frequencies of the various alleles at 8 TNF polymorphic sites in 3 groups are shown in Table 1. The frequency of TNFa10 allele was significantly higher in patients with CAG than in healthy controls (19.81% *vs* 11.89%, $P=0.04$, OR=1.83, 95%CI

Table 1 Distribution frequency of TNF alleles at 8 polymorphic loci in CAG, gastric adenocarcinoma and healthy controls

TNF locus	Allele	Size (bp)	Controls (*n*=164)	CAG (*n*=53)	Gastric adenocarcinoma (*n*=56)	*P*
TNFa	a1	97	0.0183	0.0094	0.0089	
	a2	99	0.1583	0.1698	0.2054	
	a3	101	0.0000	0.0094	0.0268	
	a4	103	0.0061	0.0094	0.0178	
	a5	105	0.0396	0.0378	0.0089	
	a6	107	0.3811	0.3585	0.3928	
	a7	109	0.0793	0.0378	0.0536	
	a8	111	0.0061	0.0094	0.0000	
	a9	113	0.0335	0.0566	0.0152	
	a10	115	0.1189	0.1981*	0.1161	0.040
	a11	117	0.0945	0.0660	0.0803	
	a12	119	0.0091	0.0000	0.0000	
	a13	121	0.0549	0.0378	0.0152	
TNFb	b1	125	0.1006	0.0755	0.1339	
	b2	126	0.0000	0.0000	0.0000	
	b3	127	0.1128	0.0943	0.0536	
	b4	128	0.3293	0.3585	0.3482	
	b5	129	0.4512	0.4717	0.4643	
	b6	130	0.0000	0.0000	0.0000	
	b7	131	0.0061	0.0000	0.0000	
TNFc	c1	159	0.2104	0.7264	0.7679	
	c2	161	0.7896	0.2736	0.2321	
TNFd	d1	124	0.0061	0.0094	0.0000	
	d2	126	0.0548	0.0755	0.0536	
	d3	128	0.0457	0.0566	0.0446	
	d4	130	0.3537	0.3774	0.3393	
	d5	132	0.1341	0.1226	0.1786	
	d6	134	0.3018	0.2925	0.2857	
	d7	136	0.0000	0.0000	0.0000	
	d8	138	0.1037	0.0660	0.0982	
TNFe	e1	99	0.0640	0.0755	0.0000	
	e3	103	0.8110	0.8302	0.7768	
	e4	105	0.1159	0.0660	0.1250	
TNF-α 308	1		0.9329	0.9434	0.9732	
	2		0.0671	0.0566	0.0268	
TNF-β Nco1	1		0.4817	0.4528	0.4464	
	2		0.5183	0.5472	0.5536	
TNF-β AspH1	1		0.3079	0.3302	0.3571	
	2		0.6921	0.6698	0.6429	

1.02-3.27). However, it was not related to age, gender, atrophic degree and intestinal metaplasia in patients with CAG (Table 2). The frequency of TNF-β Ncol*1/2 genotype was higher in patients with chronic atrophic gastritis than in healthy controls (Table 3). However no significant difference was observed (60.38% vs 46.34%, P=0.076).

The frequency of TNF-β Ncol*1/2 genotype was significantly higher in patients with gastric adenocarcinoma than in healthy controls (64.29% vs 46.34%, P=0.020, OR=2.08, 95%CI 1.12-3.86). The frequency of d2/d6 genotype was also significantly higher in patients with gastric adenocarcinoma than in healthy individuals (10.71% vs 2.44%, P=0.028, OR=4.8, 95%CI 1.18-19.47). However, TNF-β Ncol*1/2 and d2/d6 genotypes were not related to age, gender, grade of differentiation and clinicopathologic stage in patients with gastric adenocarcinoma (Table 4).

Based on maximum likelihood estimate, 4 most frequent 3-locus haplotypes have been described in 4 European populations: TNFa11b4c1, TNFa2b1c2, TNFa6b5c1, TNFa10b4c1. These haplotypes have also been observed in our study. By analysis of 2 locus association, we established 5 extended haplotypes which integrated alleles across the TNF locus in our population: TNFa6b5c1d8e4TNF308-1TNF β Nco1-1TNFAspH1-2, TNFa2b1c2d5e1TNF308-1TNF β Nco1-2TNFAspH1-2, TNFa11b4c1d4e3TNF308-1TNF β Nco1-2TNFAspH1-1, TNFa10b4c1d4e3TNF308-1TNF β Nco1-2TNFAspH1-1, TNFa2b3c1d2e3TNF308-2TNFAspH1-2. There were no significant differences in haplotype frequencies between CAG, gastric adenocarcinoma, and control groups for these haplotypes. However the frequency of TNFa6b5c1 haplotype homozygote was significantly lower in patients with gastric adenocarcinoma than in healthy individuals (1.79% vs 15.85%, P=0.006). It was not related to age, gender, grade of differentiation and clinicopathologic stage in patients with gastric adenocarcinoma.
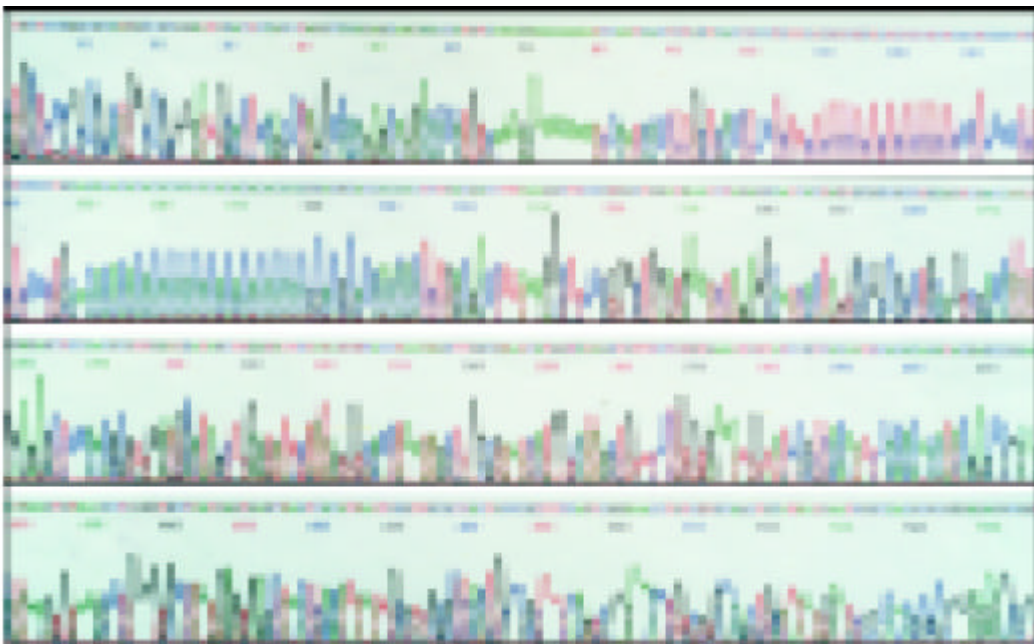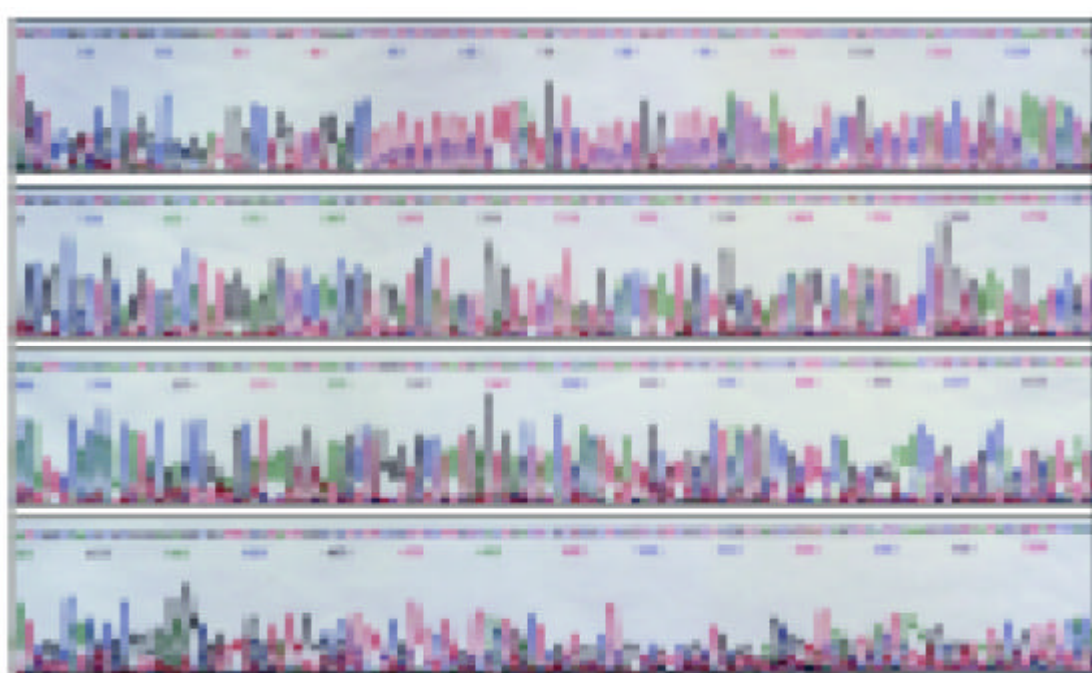

Figure 1 Nucleotide sequence of the cloned PGEM-T vector comprising TNFa6 allele (135→241) and TNFb5 allele (29→157), 150→179 fragment is 15 (AC/GT) repeats, 107→127 fragment is 10.5 (TC/GA) repeats.
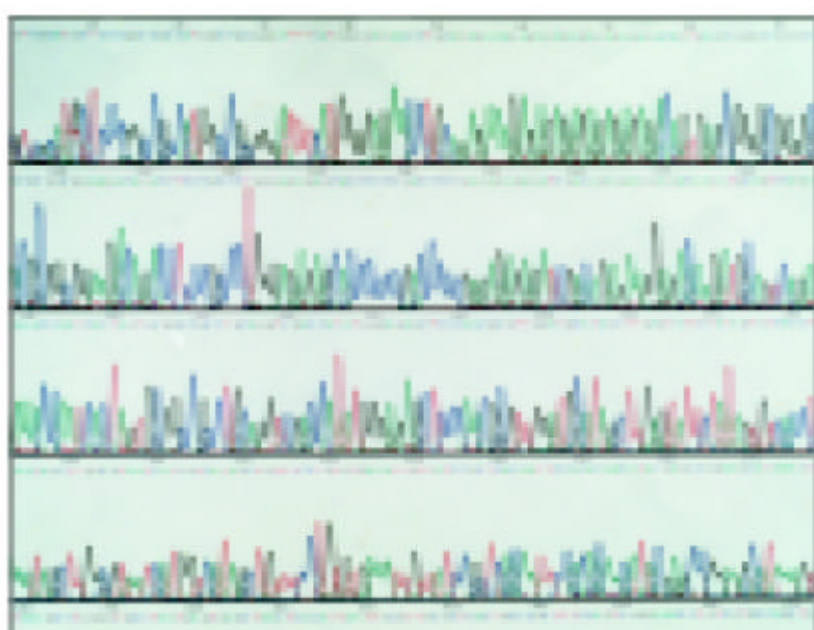
Figure 2 Nucleotide sequence of the cloned PGEM-T vector comprising TNFc1 allele (35→193), 59→76 fragment is 9 (TC/GA) repeats.
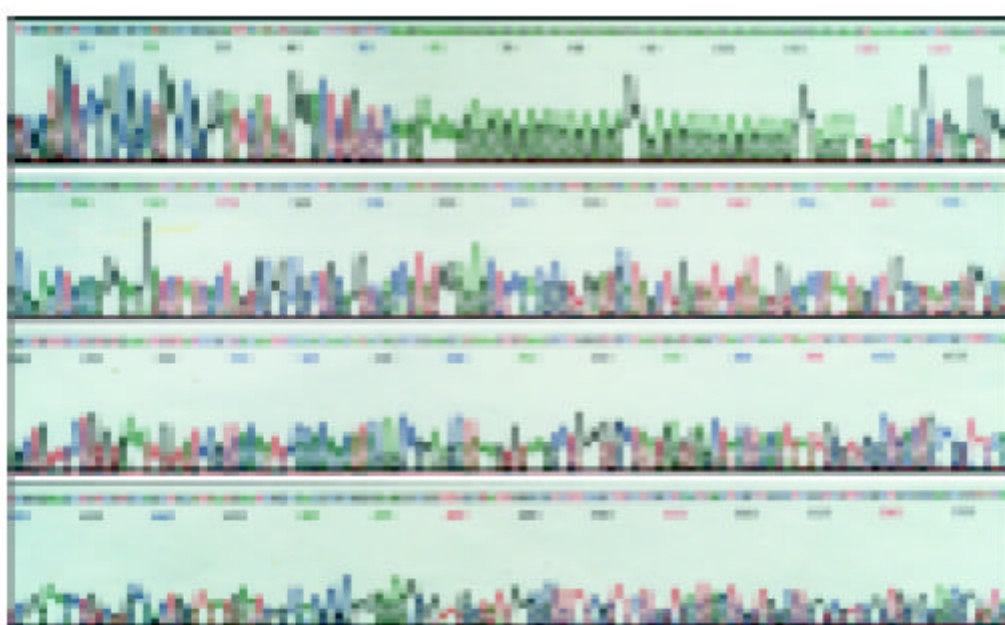
Figure 3 Nucleotide sequence of the cloned PGEM-T vector comprising TNFd4 allele (34→163), 64→85 fragment is 11 (TC/GA) repeats.

Figure 4 Nucleotide sequence of the cloned PGEM-T vector comprising TNFe3 allele (29→131), 47→62 fragment is 8 (TC/GA) repeats.

Table 2 Association between TNFa10 allele and clinical features of CAG

Groups	Age	Gender		Atrophic degree			Intestinal metaplasia	
	(yr)	M	F	Mild	Moderate	Severe	Positive	Negative
TNFa10+	53.9±9.6	13	8	3	12	6	14	7
TNFa10-	53.2±12.5	19	13	6	18	8	22	10

Table 3 Distribution frequency of TNF TNF-β Ncol*1/2 and TNFd2/d6 genotypes in CAG, gastric adenocarcinoma and healthy controls

Group	TNF-β Ncol*1/2		TNFd2/d6	
	+	-	+	-
Controls	76	88	4	160
CAG	32[a]	21	2	51
Gastric adenocarcinoma	36[a]	20	6[c]	50

[a]$P<0.05$ *vs* compared with control group of TNF-β Ncol*1/2, [c]$P<0.05$ *vs* compared with control group of TNFd2/d6.

Table 4 Association between d2/d6 and TNF-β Ncol*1/2 genotypes and clinical features of gastric adenocarcinoma

Group	Age	Gender		Grade of differentiation		Clinicopathologic stage	
	(yr)	M	F	High and moderate	Low	I-II	III-IV
d2/d6+	59.7±9.5	5	1	0	6	4	2
d2/d6-	55.1±12.5	38	12	13	37	21	29
1/2+	56.7±12.2	26	10	9	27	15	21
1/2-	53.6±11.5	17	3	4	16	10	10

The sequences of five TNF microsatellites (Figures 1-4) were consistent with that from a GeneBank database. However, several base exchanges were noted as following: TNFa at position 218 T→G, TNFc at 129 G→C, 170 A→G, TNFd at 87 G →A, 91 A→G, and TNFe at 81 C→T.

DISCUSSION

Genetic predisposition appears to be important in the pathogenesis of gastric adenocarcinoma. Genetic factors determining cancer risk have been postulated for the last decades and seem to be more apparent for gastric adenocarcinoma[16-21]. The relevant genes mediating the risk of gastric adenocarcinoma have not been identified until now. Progress of CAG towards gastric adenocarcinoma seems to be influenced by genetic factors[22,23].

A diallelic TNF-β polymorphism detected using the enzyme Ncol influences the TNF-α and/or -β secretion of peripheral blood mononuclear cells[24]. Park et al.[25] found that TNF-β Ncol*1/1 genotypes showed an increased risk for colorectal cancer, and that TNF-β*1 allele played some role in the initial step of tumorigenesis or activation of dormant tumor cells, whereas TNF-β*2 allele mediated some functions associated with cytotoxicity of tumor cells. Our result showed that the frequency of TNF-β Ncol*1/2 genotype was significantly higher in patients with gastric adenocarcinoma than in healthy individuals. It suggested that TNF-β Ncol*1/2 genotype was related to the pathogenesis of gastric cancer. At the same time we found the frequency of TNF-β Ncol*1/2 genotype was also high in patients with CAG. However, the association between TNF-β Ncol*1/2 genotype and CAG did not show a significant difference. Further studies are necessary to elucidate If TNF-β Ncol*1/2 genotype is a risk factor for CAG. The frequency of d2/d6 genotype was also significantly higher in patients with gastric adenocarcinoma than in healthy individuals. It indicated that d2/d6 genotype might have some effect on pathogenesis of gastric adenocarcinoma.

Azuma et al.[23] reported that the DQA1*0102 might contribute to resistance against H pylori associated gastric atrophy and immunogenetic factors were important in the etiology of H pylori associated gastric atrophy. Proinflammatory IL-1beta polymorphisms are associated with hypochlorhydria and atrophic gastritis in Japan[24]. The presence of the IL-1*C allele may also indicate a risk of mucosal atrophy of the stomach in the Japanese population[26]. Our study has shown an association between TNFa10 allele and CAG, but not between TNFa10 allele and gastric adenocarcinoma. It suggested that TNFa10 allele might be a host risk factor for CAG. However, it was not related to age, gender, atrophic degree and intestinal metaplasia in patients with CAG.

A significant reduction in high expressing haplotypes was found in patients with follicular lymphoma[27]. Hajeer et al.[28] reported the TNF a2-b4-d5 haplotype was significantly associated with the number of basal cell carcinoma (BCC) lesions. It provides the evidence that TNF microsatellite haplotype may influence the pathogenesis of multiple BCC. We found negative association between TNFa6b5c1 haplotype homozygote and gastric adenocarcinoma. The frequency of

TNFa6b5c1 haplotype homozygote was lower in patients with gastric adenocarcinoma. It suggested that TNFa6b5c1 haplotype homozygote might contribute to the resistance against gastric adenocarcinoma. The absence of TNFa6b5c1 haplotype homozygote might increase the risk of gastric adenocarcinoma.

The TNF-α 308A allele has been associated with enhanced TNF-α expression[10]. Machado et al. proposed that individuals carrying TNF-α 308A allele had an increased risk for gastric cancer with an OR of 1.9[29]. However our results showed that there was no association between TNF-α 308 allele and gastric adenocarcinoma. Similarly, Wu et al. reported no association was noted between gastric cancer and controls in the distribution of TNF-α genotypes in Taiwanese Chinese population[30]. The discrepancy may be attributable to ethnic difference.

Onishi et al.[31] demonstrated a significantly favorable prognosis in the renal carcinoma patients with TNF-β1/1 homozygote compared with other zygotes of TNF-beta polymorphism. The patients with TNF-β1/1 homozygote showed much lower stage and/or grade than those of other zygotes. TNF polymorphisms TNF+488A and TNF-859T were associated with grade of tumour in patients with bladder cancer[32]. However, in our study TNF-β Ncol*1/2 and d2/d6 genotypes and TNFa6b5c1 haplotype were not related to age, gender, grade of differentiation and clinicopathologic stage in patients with gastric adenocarcinoma. Patients with d2/d6 genotype were present with a lowly differentiated tumor, however the difference did not reach statistical significance. Larger sample studies are needed.

The association between TNF genetic polymorphisms and CAG and gastric adenocarcinoma is not clear. However, 2 distinct potential mechanisms can be proposed. First, TNF alleles may be involved in genetically controlled variations in TNF production as previously described. Another possible explanation is that these genes are not responsible for the pathophysiological mechanisms but are linked closely to other responsible genes. TNF has been reported to be in linkage disequilibrium with HLA genes[33-35]. The negative association of TNFa3-e1 with rheumatoid arthritis may be secondary to the negative linkage disequilibrium between TNFa3-e1 and HLA-DR4[36]. Additional studies will be necessary to investigate whether TNF genes are independent of other linked genes to play a role.

With regard to several base exchanges in our sequencing result, more studies should be done to verify whether they are caused by ethnic difference or other factors.

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