Ethnic and Gender Differences in Genetic Polymorphisms of Tumor Necrosis Factor (TNF)- α in a Japanese Population

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Abstract

Background: Tumor necrosis factor (TNF) blockers are highly effective in RA treatment, have been used widely for rheumatoid arthritis (RA) therapy worldwide, and can result in remission in combination with other disease-modifying antirheumatic drugs. However, it was reported that there are large interindividual differences in therapeutic responses to TNF blockers. In addition, there are few studies comparing ethnic and gender differences in allele frequencies of TNF- α polymorphisms. To obtain fundamental information for the establishment of personalized pharmacotherapy with TNF blockers, we investigated allele and genotype frequencies and gender differences in TNF- α polymorphisms among healthy Japanese adults and RA patients, and ethnic differences by comparing literature data.

Methods: We genotyped 292 healthy Japanese adults and 153 Japanese RA patients for TNF- α polymorphisms (TNF -238 G>A, -308 G>A, -857C>T) using the polymerase chain reaction method. Pearson's chi-square test was used to compare the allele and genotype frequencies of TNF- α polymorphisms among gender and ethnic groups.

Results: In healthy Japanese adults and RA patients, there were no associations between TNF- α genotypes and gender differences. However, we found a significant difference in the allele and genotype frequencies of TNF- α -308 G>A and -857C>T between a Japanese population and Caucasians.

Conclusion: The study showed a significant difference in the allele and genotype frequencies of TNF- α -308 G>A and -857C>T between a Japanese population and Caucasians, but there were no associations between TNF- α genotypes and gender differences in healthy Japanese adults and RA patients. Further study is necessary to investigate these associations in clinical studies whether the differences in allelic frequencies in ethnic groups contribute to the variations in response and toxicity of TNF blockers.

Key words: TNF- α polymorphism, allele frequency, genotype, gender difference, rheumatoid arthritis, Japanese

Background

Rheumatoid arthritis (RA) is a systemic inflammatory disease of unknown etiology, which is characterized by chronic progressive joint destruction. RA affects about 1% of the population worldwide^{1,2)} and 600,000–700,000 people (0.3–0.8%) in Japan. It is reported that joint destruction progresses most quickly during the first 1–2 years after RA onset³⁾. Once joint destruction occurs, the dysfunction becomes irreversible, and patients' quality of life (QOL) is severely impaired. Many inflammatory cytokines are involved in the progression of RA, among which tumor necrosis factor (TNF)- α plays a particularly important role in chronic joint pain^{3–5)}.

TNF blockers, which suppress the immune system by blocking the activity of TNF, have been used widely for RA therapy worldwide. TNF blockers are highly effective in RA treatment and can result in remission in combination with other disease-modifying antirheumatic drugs. Furthermore, they play a pivotal role in inhibiting the decline in QOL as TNF blockers reduce not only inflammation but also bone destruction. However, it was reported that there are large interindividual differences in therapeutic responses to TNF blockers, with approximately 30% of patients showing a poor clinical response and 20% discontinuing therapy because of side effects⁶⁾. If such individual differences could be predicted, it would be possible to prevent the unnecessary administration of TNF blockers in the early stage and save on RA treatment costs, resulting in a major medicoeconomic impact.

Currently, the cause of individual differences in the response of RA patients to TNF blockers remains unknown, but an association with TNF- α polymorphisms has been suggested. Various genetic polymorphisms have been reported in the promoter region of the TNF- α gene. In particular, there were many reports on TNF- α -308 G>A, and patients with TNF- α -308 G/G have a better therapeutic response to TNF blockers than patients with the A allele^{7–9)}. A meta-analysis combining six studies showed that patients with the TNF- α -308 A allele had a poorer response to TNF blocker therapy than those with the G allele¹⁰⁾. In contrast, RA patients with the TNF- α -857 T

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Manuscript received November 16, 2016; revised December 14, 2016 and December 17, 2016; accepted December 19, 2016

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allele showed a better response to etanercept therapy than those with homozygotes for the C allele¹⁰⁾. Therefore, it is suggested that TNF- α polymorphisms could be useful in predicting the treatment response to TNF blockers. It was also reported that for all outcome criteria, including a high DAS28 value for disease remission and health assessment questionnaire (HAQ) >1 for disability remission as the main predictors, female gender was associated with a high risk of nonresponse in an Italian group receiving anti-TNF treatment for RA¹¹⁾.

However, little is known about ethnic and gender differences in allelic frequencies of TNF- α polymorphisms in healthy adults and RA patients, and there are few studies on the frequencies of TNF- α polymorphisms in Japanese. If there are ethnic and gender differences in TNF genotypes, they may be associated with the difference in the therapeutic response to TNF blockers among ethnic populations or between genders.

The aim of this study was to investigate allele and genotype frequencies and gender differences in TNF- α polymorphisms among healthy Japanese adults and RA patients, and ethnic differences by comparing literature data, to obtain fundamental information for the establishment of personalized pharmacotherapy with TNF blockers.

Participants and Methods

Participants

Unrelated Japanese adult volunteers who were confirmed to be healthy in recent medical checkups and Japanese patients diagnosed with RA according to the 1987 American College of Rheumatology criteria¹²⁾ and who regularly visited the PS Clinic (Fukuoka, Japan) from December 2010 to March 2011 were enrolled in this study. All were 20 years of age or older. The study protocol was approved by the Institutional Review Board of the LTA Clinical Pharmacology Center, Fukuoka, Japan, and written informed consent was given by all volunteers and patients prior to enrollment.

Genotype determination

DNA extraction

For genetic analysis, 5 mL of peripheral blood was collected from all healthy volunteers and RA patients using the standard venipuncture technique in tubes containing ethylenediaminetetraacetic disodium salt and stored at -20°C until DNA extraction. DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA) following the manufacturer's instructions. Total genomic DNA was quantified and its purity and integrity were analyzed using the GE Healthcare GeneQuant 1300 Spectrophotometer (Fisher Scientific, Pittsburgh, PA, USA).

Genotyping of alleles of TNF- α polymorphisms

Genotyping of alleles of the TNF- α polymorphisms $-238\text{ G}>\text{A}$ (rs361525), $-308\text{ G}>\text{A}$ (rs1800629), and $-857\text{ C}>\text{T}$ (rs1799724) was performed using the TaqMan SNP Genotyping Assay from Applied Biosystems (Foster City, CA, USA) with fluorogenic binding probes. PCR amplification with the real-time PCR method was performed in 10 μL of reaction mixture including genomic DNA 8 ng, 40 $\times$ TaqMan SNP Genotyping Assay Mix (C_2215707_10, C_7514879_10, C_11918223_10) 0.252 μL , and TaqMan Universal PCR Master Mix 5 μL . The

reaction conditions for PCR were: initial denaturation for 10 min at 95°C ; 40 cycles at $92^{\circ}\text{C}/15\text{sec}$; and annealing and extension for 1 min at 60°C . The PCR system used was the StepOnePlus real-time PCR system (Applied Biosystems).

Literature search

We searched for articles that assessed TNF- α polymorphisms ($-238\text{ G}>\text{A}$, $-308\text{ G}>\text{A}$, $-857\text{ C}>\text{T}$) in healthy adults or RA patients published before May 2016 using Medline, Embase, Web of Science, Japana Centra Revuo Medicina (Ichushi) (1981–May 2016), and the database of the HapMap Project (accessed in May 2016). We also collected data from the International HapMap Project on healthy adults. "TNF- α $-238\text{ G}>\text{A}$," "TNF- α $-308\text{ G}>\text{A}$," "TNF- α $-857\text{ G}>\text{A}$," "genetic polymorphism," and "rheumatoid arthritis" were used as key words in the literature search. Inclusion criteria were that the studies: 1) involved healthy adults or RA patients; 2) assessed TNF- α $-238\text{ G}>\text{A}$, $-308\text{ G}>\text{A}$, or $-857\text{ C}>\text{T}$; 3) gave descriptions of ethnic groups, studies that mentioned the terms "Caucasian," "Han Chinese," "Mestizo," and "Japanese"; and 4) showed agreement with Hardy-Weinberg equilibrium (HWE).

Statistical analysis

Comparisons among healthy adults and RA patients in the distribution of the genotype and haplotypes, and allele frequency of ethnic and gender differences were evaluated using the chi-square test. Tests for HWE were also carried out using the chi-square test. A p-value of <0.05 was considered to represent a statistically significant difference in all analyses. All statistical analyses were performed using IBM SPSS Statistics version 23 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Participants

A total of 292 unrelated healthy Japanese adult volunteers (142 men and 150 women, median age 29 (range 20–79) years) and a total of 153 unrelated Japanese RA patients (40 men and 113 women, median age 57 (33–69) years) were enrolled in this study.

TNF- α genotype frequencies and allelic frequencies in healthy Japanese adults and RA patients and gender differences

Table 1 shows the distribution of genotype and allelic frequencies of TNF- α $-238\text{ G}>\text{A}$, $-308\text{ G}>\text{A}$, and $-857\text{ C}>\text{T}$ by gender in healthy Japanese adults and RA patients. The proportions of genotypes at each site were generally in agreement with HWE and followed Mendelian principles. No statistically significant differences in the distribution of the TNF- α genotype between the healthy and RA groups were found. Gender differences were not found in the allelic frequencies of all TNF- α polymorphisms examined in either healthy Japanese adults or RA patients.

Comparison of the distribution of haplotype patterns of TNF- α genotypes between a healthy Japanese population and Japanese RA patients

Table 2 shows 6 haplotype patterns of TNF- α -238 , -308 , and -857 in the healthy Japanese population and in Japanese RA patients. The most common of haplotype patterns were TNF-

Table 1 Frequencies of TNF- α polymorphisms in healthy Japanese adults and RA patients

	n	Genotype frequency, n (%)			p-value	Allele frequency (%)		p-value	HWE
Healthy volunteers									
TNF- α - 238		G/G	G/A	A/A		G	A		
Total	292	285 (97.6)	7 (2.3)	0 (0.0)	0.648	98.8	1.2	0.650	0.836
Men	142	138 (97.2)	4 (2.8)	0 (0.0)		98.6	1.4		0.865
Women	150	147 (98.0)	3 (2.0)	0 (0.0)		99.0	1.0		0.902
TNF- α - 308		G/G	G/A	A/A		G	A		
Total	292	285 (97.6)	7 (2.3)	0 (0.0)	0.222	98.8	1.2	0.216	0.836
Men	142	137 (96.5)	5 (3.5)	0 (0.0)		98.2	1.8		0.831
Women	150	148 (98.7)	2 (1.3)	0 (0.0)		99.3	0.7		0.934
TNF- α - 857		C/C	C/T	T/T		C	T		
Total	292	193 (66.1)	86 (29.5)	13 (4.4)	0.342	80.8	19.2	0.758	0.393
Men	142	93 (65.5)	45 (31.7)	4 (2.8)		81.3	18.7		0.601
Women	150	100 (66.7)	41 (27.3)	9 (6.0)		80.3	19.7		0.098
RA patients									
TNF- α - 238		G/G	G/A	A/A		G	A		
Total	153	148 (96.7)	5 (3.3)	0 (0.0)	0.751	98.3	1.7	0.753	0.837
Men	40	39 (97.5)	1 (2.5)	0 (0.0)		98.7	1.3		0.936
Women	113	109 (96.5)	4 (3.5)	0 (0.0)		98.2	1.8		0.848
TNF- α - 308		G/G	G/A	A/A		G	A		
Total	153	152 (99.3)	1 (0.7)	0 (0.0)	0.511	99.7	0.3	0.551	0.968
Men	40	40 (100.0)	0 (0.0)	0 (0.0)		100.0	0.0		—
Women	113	112 (99.1)	1 (0.9)	0 (0.0)		99.6	0.4		0.962
TNF- α - 857		C/C	C/T	T/T		C	T		
Total	153	91 (59.5)	54 (35.3)	8 (5.2)	0.171	77.1	22.9	0.101	0.998
Men	40	27 (67.5)	13 (32.5)	0 (0.0)		83.8	16.2		0.220
Women	113	64 (56.6)	41 (36.3)	8 (7.1)		74.8	25.2		0.685

TNF : tumor necrosis factor ; HWE : p-value of chi-square test for agreement with HWE.
p-value : comparison of men and women.

Table 2 Comparison of the distribution of haplotype patterns of TNF- α genotypes between Japanese healthy population and RA patients

Haplotypes	RA patients	Healthy volunteers	p-value
	n (%)	n (%)	
TNF- α -238, -308, -857			
G/G, G/G, C/C	88 (57.5)	182 (62.3)	0.663
G/G, G/G, C/T	51 (33.3)	83 (26.4)	
G/G, G/G, T/T	8 (5.2)	13 (4.5)	
G/G, G/A, C/C	1 (0.7)	7 (2.4)	
G/A, G/G, C/C	2 (1.3)	4 (1.4)	
G/A, G/G, C/T	3 (2.0)	4 (1.4)	

n : number of patients/healthy volunteers

α -238 G/G, -308 G/G, and -857C/C in the healthy Japanese population and Japanese RA patients. No statistically significant differences in the distribution of haplotype patterns of TNF- α genotypes were seen between the healthy Japanese population and in Japanese RA patients ($p=0.663$).

Comparison of TNF- α genotypes and allelic frequencies among ethnic groups

A total of 235 studies (MEDLINE 110 studies, Embase 125 studies) were identified in electronic searches. Among these, 9 studies of TNF- α -238 G>A^[13-21], 10 of -308 G>A^[13-15, 17, 18, 20-24] and 3 of -857C>T^[13, 15, 21] met the inclusion criteria.

Table 3 and **Table 4** show the distribution of genotypes and allelic frequencies of TNF- α -238 G>A, -308 G>A, and -857C>T in each ethnic group of healthy adults and RA

patients. The proportions of genotypes at each site in all ethnic groups were generally in agreement with HWE and followed Mendelian principles.

When comparing the TNF- α -238 G>A polymorphism in our 292 healthy Japanese adults with other healthy ethnic groups, the G allele frequency was significantly higher in Japanese than in Caucasians and Han Chinese. The G allele frequency of TNF- α -308 G>A was also significantly higher in our Japanese population than in Caucasian, Han Chinese, and Mestizo populations, and that of TNF- α -857C>T was significantly higher in Japanese in the present study than reported in Caucasians.

Comparing our 153 RA patients with those in other ethnic groups for the TNF- α -238 G>A polymorphism, the G allele frequency of TNF- α -238 G>A was significantly higher in our

Table 3 Comparison of frequencies of the TNF- α genotype and alleles among ethnic groups in healthy adults

Ethnic group	n	Genotype frequency, n (%)			p-value	Allele frequency (%)		p-value	HWE
TNF- α - 238									
		G/G	G/A	A/A		G	A		
Japanese (our study)	292	285 (97.6)	7 (2.4)	0 (0.0)	Ref.	98.8	1.2	Ref.	0.836
Japanese ²¹⁾	575	552 (96.0)	23 (4.0)	0 (0.0)	0.222	98.0	2.0	0.226	0.625
Caucasian ^{15, 16)}	864	785 (90.9)	79 (9.1)	0 (0.0)	<0.001	95.4	4.6	<0.001	0.159
Han Chinese ^{13, 14)}	373	334 (89.5)	39 (10.5)	0 (0.0)	<0.001	94.8	5.2	<0.001	0.897
Mestizo ^{17, 18)}	269	236 (87.7)	32 (11.9)	1 (0.4)	<0.001	93.7	6.3	<0.001	0.939
TNF- α - 308									
		G/G	G/A	A/A		G	A		
Japanese (our study)	292	285 (97.6)	7 (2.4)	0 (0.0)	Ref.	98.8	1.2	Ref.	0.836
Japanese ²¹⁾	575	556 (96.7)	18 (3.1)	1 (0.2)	0.642	98.3	1.7	0.390	0.044
Japanese (HapMap)	44	42 (95.5)	2 (4.5)	0 (0.0)	0.334	97.7	2.3	0.334	0.343
Caucasian ^{15, 22, 23)}	716	498 (69.6)	201 (28.1)	17 (2.3)	<0.001	83.6	16.4	<0.001	0.534
Han Chinese ^{13, 14, 24)}	773	659 (85.3)	109 (14.1)	5 (0.6)	<0.001	92.3	7.7	<0.001	0.832
Han Chinese (HapMap)	45	42 (93.3)	3 (6.7)	0 (0.0)	0.136	96.7	3.3	0.138	0.143
Mestizo ^{17, 18)}	262	241 (92.0)	21 (8.0)	0 (0.0)	0.003	96.0	4.0	0.003	0.499
TNF- α - 857									
		C/C	C/T	T/T		C	T		
Japanese (our study)	292	193 (66.1)	86 (29.5)	13 (4.5)	Ref.	80.8	19.2	Ref.	0.393
Japanese ²¹⁾	575	389 (67.7)	168 (29.2)	18 (3.1)	0.58	82.3	17.7	0.463	0.979
Caucasian ¹⁵⁾	457	386 (84.5)	66 (14.4)	5 (1.1)	<0.001	91.7	8.3	<0.001	0.259
Han Chinese ¹³⁾	271	197 (72.7)	64 (23.6)	10 (3.7)	0.237	84.5	15.5	0.104	0.105

TNF : tumor necrosis factor ; HWE : p-value of chi-square test for agreement with HWE.

p-value : comparison of each ethnic group and our Japanese population (Ref.).

Table 4 Comparison of frequencies of the TNF- α allele and genotypes in RA patients among ethnic groups

Ethnic group	n	Genotype frequency, n (%)			p-value	Allele frequency (%)		p-value	HWE
TNF- α - 238									
		G/G	G/A	A/A		G	A		
Japanese (our study)	153	148 (96.7)	5 (3.3)	0 (0.0)	Ref.	98.4	1.6	Ref.	0.837
Japanese ²¹⁾	387	376 (97.2)	11 (2.8)	0 (0.0)	0.492	98.6	1.4	0.491	0.777
Caucasian ^{15, 19, 20)}	1817	1684 (92.7)	131 (7.2)	2 (0.1)	0.207	96.3	3.7	0.059	0.739
Han Chinese ^{13, 14)}	819	756 (92.3)	61 (7.5)	2 (0.2)	0.138	96.0	4.0	0.044	0.515
Mestizo ^{17, 18)}	180	169 (93.9)	9 (5.0)	2 (1.1)	0.307	96.3	3.7	0.117	<0.001
TNF- α - 308									
		G/G	G/A	A/A		G	A		
Japanese (our study)	153	152 (99.3)	1 (0.7)	0 (0.0)	Ref.	99.7	0.3	Ref.	0.968
Japanese ²¹⁾	387	384 (99.2)	3 (0.8)	0 (0.0)	0.684	99.6	0.4	0.261	0.939
Caucasian ^{15, 20, 22, 23)}	596	415 (69.6)	172 (28.9)	9 (1.5)	<0.001	84.1	15.9	<0.001	0.060
Han Chinese ^{13, 14, 24)}	931	864 (92.8)	67 (7.2)	0 (0.0)	0.002	96.4	3.6	0.002	0.255
Mestizo ^{17, 18)}	183	154 (84.2)	26 (14.2)	3 (1.6)	<0.001	91.3	8.7	<0.001	0.138
TNF- α - 857									
		C/C	C/T	T/T		C	T		
Japanese (our study)	153	91 (59.5)	54 (35.3)	8 (5.2)	Ref.	77.1	22.9	Ref.	0.998
Japanese ²¹⁾	387	199 (51.4)	165 (42.6)	23 (6.0)	0.237	72.7	27.3	0.139	0.140
Caucasian ¹⁵⁾	363	314 (86.5)	48 (13.2)	1 (0.3)	<0.001	93.1	6.9	<0.001	0.555
Han Chinese ¹³⁾	367	244 (66.5)	113 (30.8)	10 (2.7)	0.317	81.9	18.1	0.078	0.471

TNF : tumor necrosis factor ; HWE : p-value of chi-square test for agreement with HWE.

p-value : comparison of each ethnic and our Japanese population (Ref.).

RA population than in Han Chinese RA patients. For the TNF- α - 308 G>A polymorphism, the G allele frequency of TNF- α - 308 G>A was significantly higher in our Japanese RA patients than in Caucasian, Han Chinese, and Mestizo RA patients. The frequency of the TNF- α - 857 T allele was also significantly higher in Japanese than in Caucasian RA patients.

Discussion

Recently, research efforts have been being directed toward personalized medicine, which will eventually result in healthcare tailored to the specific overall conditions of individual patients. Such personalized medicine considers both gender and genetic background through pharmacogenomic investigations to customize the most appropriate therapy to individuals, leading to improved cost-effectiveness of treatment. We therefore investi-

gated the frequency differences in TNF- α polymorphisms in unrelated healthy Japanese adults and patients with RA in comparison with several ethnic groups, to obtain fundamental information for the establishment of personalized pharmacotherapy with TNF blockers.

In terms of the distribution of genotype and allelic frequencies of TNF- α - 238 G>A, -308 G>A, and -857C>T in healthy Japanese adults and RA patients by gender, participants in the present study were generally in agreement with HWE and followed Mendelian principles, and thus we considered this an appropriate population for the pharmacogenetic/genomic investigation of TNF- α polymorphisms in Japanese. In a comparison of our results with those of a previous Japanese study²¹⁾ of allelic frequencies of TNF- α polymorphisms in both healthy Japanese adults and RA patients, there were no significant differences in

any of the polymorphisms examined. This study involved a smaller sample size than that of Seki et al.²¹⁾, but they did not investigate the ethnic and gender differences between Japanese and other ethnic groups/populations. This point differed between our study and that by Seki et al.²¹⁾. However, Li et al.²⁴⁾ reported that the A allele frequencies of TNF- α -308 G>A in Han Chinese were 3.6% in RA patients and 10.5% in healthy individuals, and in men the rates were 2.8% and 15.6%, respectively. A allele frequencies of TNF- α -308 G>A were significantly higher in the healthy than in the RA group. Soliman et al.²⁵⁾ reported that female gender was significantly associated with lower disease remission in 540 rituximab-treated patients who had experimented at least one TNF-blocker therapy failure. Although gender was found to be a predictor of nonresponse to first-line therapy with anti-TNF drugs in an Italian population receiving anti-TNF treatment for RA¹¹⁾, there was little information on the gender differences in TNF- α polymorphisms and we also did not find any gender differences in the TNF- α polymorphisms examined in the present study in either healthy Japanese adults or patients with RA.

In terms of ethnic differences in allelic frequencies of TNF- α polymorphisms in healthy adults and RA patients, different distributions of the allelic frequencies of TNF- α -238 G>A, -308 G>A, and -857C>T were observed between our Japanese population and other ethnic groups, although their statistical significance differed and similar tendencies were seen. We believed that the statistical discrepancy in ethnic differences for TNF- α -238 G>A between healthy adults and RA patients, especially between our Japanese population and Mestizos, was due to number of study participants with the TNF- α -238 G/G and G/A genotypes. It was reported that the transcriptional activation and thereby mRNA expression increased with the A allele for TNF- α -308 G>A²⁶⁾ in an in vitro study, although another report found that there was no difference in the level of transcription between the -308 A and -308 G alleles⁴⁾. High transcriptional activation with the T allele in TNF- α -857C>T was also reported²⁷⁾. Robak et al.²⁸⁾ reported that patients with high RA activity (high RA grade) had high serum levels of TNF- α and we considered that increased mRNA expression of TNF- α is associated with RA grade. From these reports and our study results, it suggests the ethnic differences in the frequencies of TNF- α polymorphisms result in the difference of the expression level of TNF- α mRNA.

On the other hand, Zeng et al.²⁹⁾ reported a better therapeutic response in patients with the G allele of TNF- α -308 G>A, while Kang et al.³⁰⁾ found a better therapeutic response to etanercept in patients with the T allele of TNF- α -857C>T in a clinical trial. This study showed that Japanese have higher frequencies of the G/G genotype of TNF- α -308 G>A as well as the C/T and T/T genotypes of TNF- α -857C>T than Caucasians. Therefore, it is speculated that the frequency of these genotypes in Japanese who show a good response to anti-TNF- α therapy is higher than in other ethnic groups, but we could not investigate the association between therapeutic response and TNF- α polymorphisms in this study because of a lack of data.

TNF- α haplotypes combined with the TNF- α -238 G>A, -308 G>A, and -857C>T genotypes in healthy Japanese adults and RA patients consisted of 6 patterns: G/G, G/G, C/C; G/G, G/G, C/T; G/G, G/G, T/T; G/G, G/A, C/C; G/A,

G/G, C/C; and G/A, G/G, C/T. Among them, the -238 G/G, -308 G/G, and -857C/C haplotypes were observed most frequently. In addition, those with the TNF- α -857 T/T genotype were always classified as the G/G, G/G, T/T haplotype. Meceli-Richard et al.³¹⁾ reported that there were five haplotypes of TNF- α (-238 A>G, -308 A>G, -857C>T) (GGC, GAC, GGT, AGC, AAC) in a cohort of 380 French Caucasians. The AAC haplotype occurred in only one person, making it extremely rare, and it was absent in our study population. A poor response to adalimumab was observed in patients with TNF- α -308 G/G, -238 G/G, and -857C/C²⁴⁾. Therefore, it might be necessary to consider the haplotypes of TNF- α (-238 A>G, -308 A>G, -857C>T) genotype to develop personalized medicine.

This study had the limitation that we could not investigate the association between TNF- α genotypes/haplotypes and therapeutic response and/or adverse drug reactions to anti-TNF- α therapy because we lacked the necessary data.

Conclusion

The study showed a significant difference in the allele and genotype frequencies of TNF- α -308 G>A and -857C>T between a Japanese population and Caucasians, but there were no associations between TNF- α genotypes and gender differences in healthy Japanese adults and RA patients. The differences in allelic frequencies in different ethnic groups in healthy adults and RA patients may contribute to the variations in response and toxicity of TNF blockers, but further study is necessary to investigate these associations in clinical studies.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the Clinical Pharmacology Center, Sumida Hospital, and PS Clinic, and written informed consent was given by all volunteers and patients prior to enrollment.

Funding

This study was supported in part by Grants for Encouragement of Research from the Japan Research Foundation for Clinical Pharmacology.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

JH, MH, and MS carried out the genotype determinations. TT, TT, and SI carried out the clinical study. JH, MH, and MS performed the statistical analyses. JH, MH, TT, TT, SI, MS, and MM participated in study design and coordination and drafted the manuscript. All authors read and approved the final manuscript.

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