Tumor necrosis factor alpha -308 gene polymorphism in patients with anorexia nervosa

Nuray Kanbur[1], Lütfiye Mesci[2], Orhan Derman[1], Tuğba Turul[2], Füsun Çuhadaroğlu[3] Tezer Kutluk[1], İlhan Tezcan[2]

Sections of [1]Adolescent Medicine, [2]Immunology, Department of Pediatrics, and [3]Department of Child and Adolescent Psychiatry, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Kanbur N, Mesci L, Derman O, Turul T, Çuhadaroğlu F, Kutluk T, Tezcan İ. Tumor necrosis factor alpha -308 gene polymorphism in patients with anorexia nervosa. Turk J Pediatr 2008; 50: 219-222.

Tumor necrosis factor alpha (TNF-α) is a principal cytokine that may induce weight loss. TNF-α -308 G to A polymorphism increases transcription of TNF-α in vitro. The aim of this study was to investigate whether TNF-α gene promoter polymorphism at position -308 (G to A substitution) is one of the factors playing a role in the development of anorexia nervosa (AN). Sixteen patients with AN, aged 11-20 years, were included in this study, and 5/16 (31%) patients had TNF-α –308 G/A genotype. In the control group, 12/174 (7%) had -308 G/A genotype. There was a significant statistical difference between the patient and control groups (p=0.007). The minimum body mass index (BMI) values ever recorded for each patient during the course of the disease were significantly higher in the five patients with TNF-α -308 G to A polymorphism (p= 0.003). TNF-α gene promoter polymorphism at position –308 might be associated with a predisposition to AN and initiate the disease. The protective mechanisms that affect clinical manifestation of the disease may be related with other anti-inflammatory cytokines or immunologic mechanisms.

Key words: anorexia nervosa, tumor necrosis factor-α, promoter polymorphism.

Anorexia nervosa (AN) is a serious eating disorder characterized by extreme, self-engendered weight loss, usually seen in adolescent girls and less commonly in prepubertal children and middle-aged women or men[1,2]. The cause is elusive, with social, psychological, and biological processes all seeming to play a major part[3,4].

Cytokines released from immune cells may participate in communication between the immune system and the central nervous system. Thus, cytokines might play important roles in the etiology of AN and the pathogenesis of the associated medical complications[5]. Tumor necrosis factor-α (TNF-α), or cachectin, is a principal cytokine that may induce weight loss in malignancies and certain chronic infections[6]. It has also been shown that repeated administration of sublethal doses of TNF-α to rats rapidly induces anorexia, resulting in cachexia and depletion of lipid and protein stores[7].

Recent studies have indicated direct associations between polymorphism in cytokine gene promoter sequences and levels of mRNA or protein production[8-10]. A G to A substitution at position -308 in the promoter of the TNF-α gene increases in vitro transcription of TNF-α by approximately 6- to 9- fold[10].

The aim of this study was to investigate TNF-α gene promoter polymorphism at position -308 (G to A substitution) in patients with AN.

Material and Methods

Subjects

Sixteen patients with AN aged 11-20 years were included in this study. Except for one, all were females. Both in-patients and out-patients with the diagnosis of AN were included in the study. All patients fulfilled the Diagnostic and Statistical Manual of Mental Disorders –IV (DSM-IV) criteria for AN (American Psychiatric

Association, 1994)[11]. The sex, age, age at onset of illness, minimum body mass index (BMI), type of AN (restricting, purging), and clinical follow-up (in- or out-patient) of the patients are seen in Table I.

Table I. Characteristics of Anorexia Nervosa Patients According to Genotypes in the TNF-α Gene Promoter Region at Position –308

Patient No.	Gender	Age	Age at onset of illness	Type of AN	Clinical follow-up	Minimum BMI	Genotype of -308 TNF-α gene promoter
1	F	17	15	Restricting	In-patient	11.47	GG
2	F	14	12	Restricting	In-patient	11.57	GG
3	F	11	11	Restricting	Out-patient	13.07	GG
4	F	14	14	Restricting	In-patient	13.30	GG
5	M	12	12	Restricting	In-patient	13.33	GG
6	F	16	15	Restricting	In-patient	13.51	GG
7	F	17	15	Restricting	In-patient	14.09	GG
8	F	14	14	Restricting	Out-patient	15.05	GG
9	F	20	18	Purging	In-patient	15.57	GG
10	F	20	18	Purging	Out-patient	16.33	GG
11	F	16	16	Restricting	Out-patient	16.53	GA
12	F	15	14	Purging	Out-patient	17.10	GG
13	F	17	15	Restricting	Out-patient	18.51	GA
14	F	16	15	Purging	Out-patient	18.73	AA
15	F	17	15	Purging	In-patient	18.75	AA
16	F	16	14	Restricting	Out-patient	18.97	GA

The first control group consisted of 14 healthy Turkish adolescents (2 males, 12 females) aged 11-20 years. A second, larger, control group consisting of 174 healthy subjects from a Turkish adult population was also used in this study because prevalence of gene polymorphisms varies among populations.

After written informed consent was obtained, 5 ml peripheral venous blood was drawn from each individual into a tube containing EDTA as anticoagulant.

TNF-α -308 polymorphism genotyping

Restriction fragment length polymorphism (RFLP) method was used to identify TNF-α -308 gene promoter polymorphism.

Genomic DNA was extracted from mononuclear cells of peripheral blood using the QIAamp DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany). TNF-α promoter region genotyping was performed by polymerase chain reaction (PCR) followed by restriction enzyme digestion. PCR amplification for the –308 G/A polymorphism was carried out using the forward primer 5'–AGGCAATAGGTTTTGAGGGCCAT-3' and the reverse primer 5'–TCCTCCC CCTGCTCCGATTCCG-3', followed by digestion with the restriction enzyme (RE) NcoI. The reaction mixtures of PCR were incubated for 2 min at 95°C, followed by 35 cycles of denaturing at 94°C for 1 min, annealing at 60°C for 1 min and final elongation at 72°C for 5 min.

The amplified segments were controlled in 1% agarose gel. The product of PCR was observed to be 107 bp. Digestions for the REs were performed at 37°C overnight. The digestion products were analyzed in ethidium bromide-stained 3% agarose gel under ultraviolet light.

TNF genotype is defined as TNF G/G or TNF-α 1, whereas TNF polymorphism at position –308 (G→A substitution) results in a TNF (A/G) or TNF (A/A) defined as TNF-α 2.

Statistics

Fisher's exact test was used to compare the ratio of subjects having –308 G/A polymorphism within the patient and control groups. Pearson chi-square test was used to compare the allele frequencies of the patient and control groups. Mann-Whitney U test was used to evaluate the statistical difference of minimum BMI values of the patients having and not having G/A polymorphism within the patient group.

Results

The genotypes of the AN patients in the TNF-α gene promoter region at position –308 are seen in Table I. Out of 16 AN patients, 11 had GG (68.7%), 3 had GA (18.7%) and 2 had AA (12.5%) genotypes; 31% of patients had G to A substitution at position -308 in promoter of the TNF-α gene.

Out of 174 healthy control subjects from a Turkish adult population, 162 had GG (93%), 10 had GA (6%) and 2 had AA (1%) genotypes; 7% of the second control group had -308 GA/AA genotype polymorphisms (p=0.007).

In the age-matched healthy control group, out of 14 subjects, 12 had GG (85.7%), 2 had GA (14.2 %) and none had AA haplotypes; 14% of controls had polymorphism (p=0.256).

The allele frequencies in the TNF-α gene promoter region at position –308 in the patient group were 78% G and 22% A alleles. In the larger second control group, the allele frequencies were 96% G and 4% A (p=0.000). In the age-matched control group, 93% G and 7% A alleles were found (p=0.111).

When we divided the AN patients into two groups according to the type of AN, 11 patients had restricting type and 5 had purging type (Table II). Out of 11 patients with restricting type, 3 (27%) had GA mutation, while out of 5 patients with purging type, 2 (40%) had AA mutation (Table II).

The patients were ordered according to their minimum BMI values ever recorded during the course of the disease (Table I). The 5 patients having GA and AA genotypes were from among the six patients with the higher minimum BMI values. The minimum BMI values were significantly higher in the 5 patients with TNF-α -308 G to A polymorphism (u=1, p=0.003).

Discussion

Tumor necrosis factor-α activates the hypothalamic-pituitary-adrenal axis and induces suppression of food intake[12]. Furthermore, TNF-α induces insulin resistance, suppresses lipoprotein lipase activity and has a catabolic effect on energy storage tissue, inducing weight loss[12].

Increased spontaneous TNF-α synthesis from peripheral blood mononuclear cells in patients with AN was reported[13]. In addition, it is reported that patients with anorexia have higher-than-normal concentrations of TNF-α and soluble forms of TNF-α receptors in plasma, regardless of changes in body weight[5]. Recently, Kahl et al.[1] reported a significant increase in TNF-α and interleukin-6 mRNA expression in anorectic patients at admission. During the follow-up period, the expression of TNF-α mRNA remained significantly higher while interleukin-6 mRNA expression decreased[1]. Thus, TNF-α may have a role in the pathogenesis of AN and its complications.

G to A substitution at position -308 in promoter of the TNF-α gene increases production of TNF-α[10]. In this study, we investigated whether TNF-α gene promoter polymorphism at position –308 is associated with a tendency to AN.

Our results provide some evidence of association between TNF-α gene promoter polymorphism at position –308 and AN, since the frequency of high producer genotypes was found to be higher in the patient group (31%) than in the control group (7%) (p=0.007) and the A allele frequency was higher in the patient group (22%) than in the control group (4%) (p=0.000), although the patient group was small.

In this study, we divided the patients into two groups according to the classification of the DSM-IV criteria for AN. In the restricting group, all the patients with polymorphism had

Table II. Distribution of the Genotypes in the TNF-α Gene Promoter Region at Position –308 of the AN Patients According to Sub-Types and in Healthy Age-Matched Adolescents

		Genotypes		
Subjects		GG	GA	AA
Anorexia nervosa (AN) patients (n=16)	Restricting type (n=11)	8 (73%)	3 (27%)	–
	Purging type (n=5)	3 (60%)	–	2 (40%)
Healthy age-matched adolescents (n=14)		12 (86%)	2 (14%)	–

GA genotype, but in the purging group, the patients with polymorphism had AA genotype. Since the numbers in each group is not enough for statistical evaluation, we cannot draw any conclusions regarding association between different mutations and clinical sub-types.

The minimum BMI values ever recorded for each patient during the course of the disease were significantly higher in the five patients with TNF-α -308 G to A polymorphism. These data lead us to think that, although TNF-α gene promoter polymorphism at position –308 might be associated with a predisposition to AN and initiate the disease, it might also cause some other mechanisms and have pathophysiological roles that protect the patient from losing extra weight; thus, the minimum BMI values of these patients were significantly greater than of the others who did not have polymorphism. These protective mechanisms that affect clinical manifestation of the disease may be related with other suppressive and anti-inflammatory cytokines and their combination or immunologic mechanisms. In addition to genetic analysis, measurement of the circulating levels of TNF-α, interleukins (IL-1,2,4,6,10), interferon, transforming growth factor[4] and anti-inflammatory cytokines in AN will help in examining the possibility of any protective mechanism.

REFERENCES

1. Kahl KG, Kruse N, Rieckmann P, Schmidt MH. Cytokine mRNA expression patterns in the disease course of female adolescents with anorexia nervosa. Psychoneuroendocrinology 2004; 29: 13-20.
2. Coupey SM. Anorexia nervosa. In: Friedman SB, Fisher MM, Schonberg SK, Alderman EM (eds). Comprehensive Adolescent Health Care (2nd ed). St Louis: Mosby; 1998: 247-262.
3. Fairburn CG, Harrison PJ. Eating disorders. Lancet 2003; 361: 407-416.
4. Nagata T, Tobitani W, Kiriike N, Iketani T, Yamagami S. Capacity to produce cytokines during weight restoration in patients with anorexia nervosa. Psychosom Med 1999; 61: 371-377.
5. Nakai Y, Hamagaki S, Takagi R, Taniguchi A, Kurimoto F. Plasma concentrations of tumor necrosis factor–α (TNF-α) and soluble TNF receptors in patients with anorexia nervosa. J Clin Endocrinol Metab 1999; 84: 1226-1228.
6. Tracey KJ, Lowry SF, Cerami A. Cachectin: a hormone that triggers acute shock and chronic cachexia. J Infect Dis 1988; 157: 413-420.
7. Tracey KJ, Wei H, Manogue KR, et al. Cachectin/tumor necrosis factor induces cachexia, anemia and inflammation. J Exp Med 1988; 167: 1211-1227.
8. Olomolaiye O, Wood NA, Bidwell JL. A novel NIaIII polymorphism in the human IL-6 promoter. Eur J Immunogenet 1998; 25: 267.
9. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997; 24: 1-8.
10. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci USA 1997; 94: 3195-3199.
11. American Psychiatric Association. Diagnosis and Statistical Manual of Mental Disorders (4th ed) (DSM-IV). Washington: American Psychiatric Association; 1994.
12. Ando T, Ishikawa T, Kawamura N, et al. Analysis of tumor necrosis factor-α gene promoter polymorphisms in anorexia nervosa. Psychiatr Genet 2001; 11: 161-164.
13. Schattner A, Steinbock M, Tepper R, Schonfeld A, Vaisman N, Hahn T. Tumour necrosis factor production and cell-mediated immunity in anorexia nervosa. Clin Exp Immunol 1990; 79: 62-66.