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Association of tumor necrosis factor receptor type 2 +587 gene polymorphism with severe chronic periodontitis

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Abstract

Background: Genetic polymorphisms for cytokines and their receptors have been proposed as potential markers for periodontal disease. Tumor necrosis factor receptor 2 (TNFR2) is one of the cell surface receptors for TNF-α. Recent studies have suggested that TNFR2 gene polymorphism is involved in autoimmune and other diseases.

Objectives: The aim of the present study is to evaluate whether TNFR2(+587T/G) gene polymorphism is associated with chronic periodontitis (CP).

Methods: One hundred and ninety-six unrelated subjects (age 40–65 years) with different levels of CP were identified according to established criteria, including measurements of probing pocket depth (PPD), clinical attachment level (CAL), and alveolar bone loss (BL). All subjects were of Japanese descent and non-smokers. Single nucleotide polymorphism at position +587(T/G) in the TNFR2 gene was detected by a polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) method.

Results: The frequency and the positivity of the +587G allele were significantly higher in severe CP patients than in controls ($p = 0.0097$; odds ratio = 2.61, $p = 0.0075$; odds ratio = 3.06). In addition, mean values of PPD, CAL, and BL were significantly higher in the +587G allele positive than in the negative subjects ($p = 0.035$, 0.022, and 0.018, respectively).

Conclusions: These findings suggest that the TNFR2(+587G) polymorphic allele could be associated with severe CP in Japanese.

Key words: chronic periodontitis; Japanese; polymorphism (genetics); tumor necrosis factor receptor

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The search for genetic markers associated with the severity, as well as the susceptibility, for periodontal disease has recently been receiving considerable attention. In particular, polymorphisms in genes encoding molecules of the host defense system such as cytokines, have been targeted as potential genetic markers (Kornman et al. 1997, Gore et al. 1998, Diehl et al. 1999, Galbraith et al. 1999).

Tumor necrosis factor (TNF) as well as interleukin (IL)-1 are proinflammatory cytokines produced by a wide spectrum of cells. These cytokines have been found at high levels in gingival crevicular fluids and in gingival tissues from periodontitis-affected sites of humans, and it has been known to induce tissue destruction and bone resorption (Wilton et al. 1992, Page et al. 1997).

TNF-α mediates its diverse biologic effects by binding two high-affinity, cell surface receptors, TNF receptor 1 (TNFR1, p55 TNFR) and TNF receptor 2 (TNFR2, p75 TNFR). Both types of TNFRs are expressed by almost all cell types, including macrophages, lymphocytes, neutrophils, and fibroblasts (Aderka 1996). TNFR1 and TNFR2 have differences in both their extracellular and cytoplasmic domains and thus are functionally distinct. TNF signaling to cells is largely mediated by TNFR1, while the main function of TNFR2 is thought to enhance this activity by binding TNF and then passing it on to the TNFR1, i.e. ligand passing. Thus, TNFR2 would increase the sensitivity of

a cell to TNF stimulation and enhance the TNFR1-mediated response. Soluble forms of TNFR2 are generated by proteolytic cleavage of the extracellular domain of TNFR2. This soluble TNFR2 can compete for TNF with the cell surface receptors and block the cytokine activity (Aderka 1996). Thus, the role of TNFR2 and soluble TNFR2 is not limited to signal transduction but includes extracellular regulatory functions affecting TNF bioavailability. The potential biological and clinical significance of these receptors has been reported in inflammatory, infectious, malignant, and autoimmune disorders such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).

With regard to periodontal disease, it has been reported that sulcular epithelial cells, monocyte/macrophage-like cells, fibroblasts, and endothelial cells express TNFR1 and TNFR2 as well as TNF-α (Tervahartiala et al. 2001). Graves et al. (1998) Assuma et al. (1998), and Delima et al. (2001) indicated that soluble TNFR2 significantly reduced the loss of connective tissue attachment and the loss of alveolar bone in experimental periodontitis. In addition, Ohe et al. (2000) exhibited that both TNF-α and IL-1β upregulated the gene expression of TNFR2 and did not affect that of TNFR1. These cytokines upregulated the release of soluble TNFR2 from human gingival fibroblasts but not that of soluble TNFR1. These findings suggest that TNFR2 may modulate TNF-α-mediated inflammatory responses in periodontal disease.

Recent reports have also been indicating that allelic variation in cytokine genes and factors influences the clinical outcome, susceptibility and progression of periodontal disease (Kornman et al. 1997, Gore et al. 1998, Kobayashi et al. 2000, 2001). We have recently reported the association of TNF-α, IL-1, and IL-10 gene polymorphisms with early onset periodontitis in Japanese (Endo et al. 2001, Tai et al. 2002, Yamazaki et al. 2001), and the results of our succeeding study have exhibited that a variable number of tandem repeat (VNTR) polymorphisms in the IL-1 receptor antagonist gene are associated with generalized early onset periodontitis (Tai et al. 2002).

The human TNFR2 gene is on chromosome 1p36 (Santee & Owen-Schaub 1996). In the TNFR2 gene, polymorphisms with amino substitution are at positions +511–512(GC/CG) within exon 4, +587(T/G) and +694 (G/A) within exon 6 and +1176 (G/A) within exon 9, and polymorphisms in the promoter region are at positions −1413(A/C), −1120(G/C) and VNTR (Santee & Owen-Schaub 1996, Tsuchiya et al. 2000). Komata et al. (1999) and Tsuchiya et al. (2000) have completed the variation screening of the entire TNFR2 coding region in Japanese subjects, suggesting that no polymorphisms in the TNFR2 gene were found at positions −1413, −1120, +511–512, and +1176. Numerous studies have reported that single nucleotide polymorphism at position +587(T/G, Met 196 Arg) (Keen et al. 1999, Komata et al. 1999) is associated with autoimmune and the other diseases, i.e. SLE (Komata et al. 1999, Morita et al. 2001), narcolepsy (Hohjoh et al. 2000), and graft-versus-host disease (Ishikawa et al. 2002) in Japanese and RA in Caucasians (Barton et al. 2001, Fabris et al. 2002). Association between this polymorphism and the response to anti-TNF-α therapy in RA patients has also been reported (Fabris et al. 2002). Moreover, it has been suggested that this amino acid substitution could influence signal transduction of TNF-α, resulting in increased IL-6 production and downregulate suppressing inflammatory responses mediated by TNFR1 (Komata et al. 1999, Morita et al. 2001). Thus, it is of particular interest to examine the association of periodontal disease with the TNFR2(+587T/G) gene polymorphism that may have a regulatory role in inflammatory responses.

Therefore, in this study, we investigated the distributions of the TNFR2 (+587T/G) gene polymorphism in Japanese subjects with different levels of chronic periodontitis (CP). Then, we analyzed whether this gene polymorphism may be responsible in part for the genetic susceptibility or severity factors to CP.

Material and Methods

Subjects and clinical assessments

One hundred and ninety-six unrelated subjects (age 40–65 years) with different levels of CP were recruited for this study, out of a total of 431 patients who had been referred to the Periodontal Clinic of Niigata University Dental Hospital and agreed to participate in this research. All participants were of Japanese descent and non-smokers as determined by a standard questionnaire. None of them had a history or current manifestation of systemic disease. The study was approved by the Institutional Review Board of the Niigata University Faculty of Dentistry, and a written informed consent was obtained from every participants before inclusion in the study in accordance with the Helsinki declaration.

All subjects were evaluated clinically and radiographically at the first visit by several periodontists to assess the following periodontal measurements: number of teeth, probing pocket depth (PPD), clinical attachment level (CAL), supragingival plaque accumulation, bleeding on probing (BOP) and alveolar bone loss (BL) as previously described and performed by Kobayashi et al. (2000).

The subjects were classified into the following three groups according to the partially modified criteria of Kornman et al. (1997) and Greenstein & Hart (2002).

(1) Severe CP: subjects having more than seven interproximal sites with ⩾50% BL and total mean BL of >34%.
(2) Moderate CP: subjects having less than three interproximal sites with ⩾50% BL and total mean BL of 16–34%.
(3) Controls: subjects having no PPDs >3 mm and no sites with BL>15%.

Clinical characteristics of the subjects are summarized in Table 1.

Extraction of DNA

Genomic DNA was obtained from peripheral blood using a DNA extraction kit (Wako Pure Chemical Industries. Inc., Osaka, Japan), and extraction procedures were done according to the manufacturer's instructions.

Genotyping of TNFR2(+587T/G) gene polymorphism (PCR-RFLP)

Sample DNA 35–70 ng was amplified in 25 μl volume of the reaction mixture containing 10 × reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, a pair of 0.75 μM each primer, and 1.25 U Taq polymerase (AmpliTaq Gold™, Applied Biosystems, Foster City, CA, USA). The polymerase chain reaction (PCR) products were analyzed by a PCR-restriction fragment length polymorph-

Table 1. Clinical characteristics of non-smoking CP patients and controls

Characteristic	Controls (n = 66)	Moderate CP (n = 72)	Severe CP (n = 58)
Age	49.3 ± 1.0	53.8 ± 0.9	48.7 ± 0.9
Male/Female	20/46	25/47	24/34
Number of teeth	27.4 ± 0.3	26.8 ± 0.3	25.4 ± 0.4
Mean PPD (mm)	2.3 ± 0.1	2.9 ± 0.1	4.3 ± 0.1
Mean CAL (mm)	2.5 ± 0.1	3.4 ± 0.1	5.3 ± 0.2
Mean BL (%)	11.5 ± 0.3	23.8 ± 0.6	46.2 ± 1.3
Sites with plaque (%)	39.4 ± 2.2	48.0 ± 2.3	50.4 ± 2.7
Sites with BOP (%)	9.9 ± 1.8	17.3 ± 2.2	30.2 ± 3.7

Values represent the mean ± standard error.

isms (RFLP) method. The primers used were as follows: sense 5′-ACT CTC CTA TCC TGC CTG CT-3′; anti-sense 5′-TTC TGG AGT TGG CTG CGT GT-3′ (Komata et al. 1999). The PCR conditions were: first denaturation for 10 min at 95°C, followed by 35 cycles of a 1 min denaturing at 95°C, a 1 min annealing at 60°C, and a 1 min extension at 72°C, and 1 cycle at 72°C for 5 min. The PCR products were checked by 2% agarose gel electrophoresis and then, digested with 5 U of Hsp92 II (Promega Corporation, Madison, WI, USA) at 37°C overnight. The restriction fragments were determined on 3% agarose gel electrophoresis, stained with ethidium bromide. The resulting products of 133+109 bp (allele1) and 242 bp (allele2) were diagnostic.

Direct sequencing

Partial samples were further confirmed by a direct sequencing method. Briefly, the nucleotide sequence of DNA was analyzed by cycle sequencing coupled with the same primers as those for RFLP analysis, followed by using BigDye Terminator Cycle Sequencing Kit and the ABI PRISM 377 DNA Sequencer (Applied Biosystems) according to the manufacturer's instructions.

Statistical analysis

The association of genotype and allele frequencies and allele positivities (% of individuals who carried at least one copy of polymorphic alleles) was determined by χ^2 and further adjusted for multiple comparisons using Bonferroni's correction, where the significance was accepted at $p<0.016$. χ^2 analysis was also used to test for deviation of genotype frequencies from Hardy–Weinberg expectations.

Differences in clinical parameter values between the TNFR2(+587G) allele positive and negative subjects were compared by the non-parametric Mann–Whitney *U*-test. A logistic regression analysis was utilized to assess the relationship between genotype and disease status while adjusting for potential confounding factors. We carried out this analysis with severe CP versus controls as dependent variable and age, gender, accumulation of plaque, and TNFR2(+587T/G) gene polymorphism as independent variables. Odds ratios were calculated with 95% confidence intervals (CI). Statistical analyses were performed by using the standard statistical software (StatView J-4.5 application program, SAS Institute Inc., Cary, NC, USA). Significance was set at 5% ($p<0.05$).

Results

We examined the distribution of TNFR2+587 genotypes in Japanese non-smoking subjects with different levels of CP as shown in Table 1. The genotype frequency in 66 controls was in Hardy–Weinberg equilibrium ($\chi^2 = 0.057$, $p>0.05$) and corresponded to those published in representative epidemiological studies of normal Japanese population (Komata et al. 1999).

We studied next whether the TNFR2 (+587T/G) gene polymorphism was associated with the severity of CP. In Table 2, the moderate or the severe CP patients group was compared with the controls. The frequency of the +587 (T/G) genotype tended to differ in the severe CP group when compared with the controls, but this did not reach a significant level after adjusting by Bonferroni's correction (3×2 contingency table; $\chi^2 = 7.15$, nominal $p = 0.028$). The frequency of the +587G polymorphic allele was significantly increased in the severe CP group (20.7% in severe CP versus 9.1% in controls, $\chi^2 = 6.69$, $p = 0.0097$; odds ratio = 2.61, 95% CI = 1.17–5.87). In addition, the positivity of the +587G allele was significantly higher in the

Table 2. Distributions of the TNFR2(+587T/G) gene polymorphism in non-smoking CP patients and controls

	Controls		Moderate CP		Severe CP		Control versus moderate		Control versus severe	
	%	n = 66	%	n = 72	%	n = 58	nominal *p*-value	OR (95%CI)	nominal *p*-value	OR (95%CI)
Genotype										
TT	**83.3**	55	**81.9**	59	**62.1**	36	NS	–	0.028	–
TG	**15.2**	10	**16.7**	12	**34.5**	20				
GG	**1.5**	1	**1.4**	1	**3.4**	2				
*Allele frequency**										
T	**90.9**	120	**90.3**	130	**79.3**	92	NS	1.08	0.0097‡	2.61
G	**9.1**	12	**9.7**	14	**20.7**	24		(0.45–2.60)		(1.17–5.87)
Polymorphic allele positivity†										
G+	**16.7**	11	**18.1**	13	**37.9**	22	NS	1.10	0.0075‡	3.06
G-	**83.3**	55	**81.9**	59	**62.1**	36		(0.42–2.90)		(1.23–7.70)

*Total number of alleles: controls $2n = 132$, moderate CP $2n = 144$, Severe CP $2n = 116$.

†Percentage of individuals who carry at least one copy of polymorphic allele (TNFR2+587G allele).

‡Significance at the level of 5% using Bonferroni's correction.

CP, chronic periodontitis; NS, not significant.

Table 3. Clinical characteristics of non-smoking CP patients based on the positivity of the TNFR2+587G allele

Characteristic	Allele G positive (n = 46)	Allele G negative (n = 150)	*p*-value
Age	50.0 ± 1.0	51.0 ± 0.7	NS*
Male/Female	18/28	51/99	NS
Number of teeth	26.0 ± 0.5	26.8 ± 0.2	NS
Sites with plaque (%)	46.6 ± 2.9	45.9 ± 1.6	NS
Sites with BOP (%)	21.1 ± 3.5	17.9 ± 1.8	NS

Values represent the mean ± standard error.
*NS: Not statistically significant between the +587G allele positive and negative subjects.

Table 4. Logistic regression analysis for an increased severity of CP

Variable	OR	95% CI	*p*-value
Age	0.98	0.93–1.03	NS*
Gender	1.68	0.74–3.80	NS*
Sites with plaque (%)	1.04	1.01–1.06	0.0016
Positivity of the TNFR2+587 G allele	3.45	1.41–8.44	0.0067

Logistic regression with disease status (0: controls, 1: severe CP) as dependent variable, independent variables: age between 40 and 65 years as continuous, gender (0: female, 1: male), sites with plaque (%) as continuous, and positivity of the TNFR2+587 G allele (0: negative, 1: positive).
*NS: Not significant.

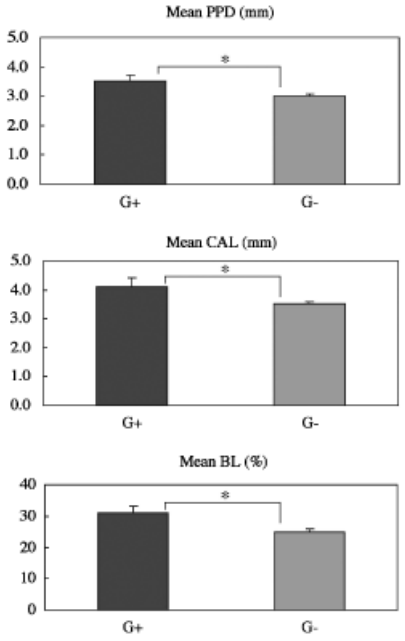

Fig. 1. Comparisons of mean values of PPD (mm), CAL (mm), and BL (%) between the +587G allele positive (G+) and negative (G−) CP patients. All of the measured values were significantly higher in the +587G allele positive than in the negative subjects (mean ± standard error: mean PPD; 3.5 ± 0.2 mm versus 3.0 ± 0.1 mm, $p = 0.035$, mean CAL; 4.1 ± 0.3 mm versus 3.5 ± 0.1 mm, $p = 0.022$ and mean BL; 30.9 ± 2.4% versus 24.9 ± 1.2%, $p = 0.018$, respectively.

severe CP group than in the controls (37.9% in severe CP versus 16.7% in controls, $\chi^2 = 7.15$, $p = 0.0075$; odds ratio = 3.06, 95% CI = 1.23–7.70). On the other hand, no significant differences in the genotype and allele frequencies, and allele positivity of the +587(T/G) gene polymorphism were found between the moderate CP group and the controls.

Clinical characteristics of the TNFR2(+587G) allele positive and negative subjects were summarized in Table 3. No significant differences were found in age, gender, number of teeth, sites with plaque (%), and sites with BOP (%) between the +587G allele positive and negative subjects. Moreover, the mean values of PPD (mm), CAL (mm) and BL (%) were compared between the TNFR2(+587G) allele positive and negative subjects, as shown in Fig. 1. All of the measured values were significantly higher in the +587G positive than in the negative subjects (mean ± standard error: mean PPD; 3.5 ± 0.2 mm versus 3.0 ± 0.1 mm, $p = 0.035$, mean CAL; 4.1 ± 0.3 mm versus 3.5 ± 0.1 mm, $p = 0.022$ and mean BL; 30.9 ± 2.4% versus 24.9 ± 1.2%, $p = 0.018$, respectively).

Both environmental and genetic factors have been considered to contribute to the pathogenesis of CP. Smoking, age, and plaque accumulation have been shown to be associated with an increased severity of CP (Grossi et al. 1995, Martinez-Canut et al. 1995). For this reason, no-smoking subjects stratified by the smoking status were included to evaluate the association of one of the genetic factors with the severity of CP in a possibly homogeneous population. In order to evaluate the association of the TNFR2(+587T/G) gene polymorphism with an increased severity of CP while adjusting for possible confounding factors, we furthermore performed a logistic regression analysis. As can be seen in Table 4, the logistic regression analysis indicated that the TNFR2(+587T/G) gene polymorphism as well as sites with plaque (%) were significant factors of an increased severity of CP. Interestingly, the TNFR2(+587T/G) gene polymorphism was found to be a highly significant factor in the model ($p = 0.0067$; odds ratio 3.45, 95%CI = 1.41–8.44). On the other hand, age and gender were not statistically significant.

Discussion

In this study, we investigated whether the TNFR2(+587T/G) gene polymorphism was associated with the severity of chronic periodontitis in Japanese non-smoking patients. When compared between the severe CP and the controls, the frequency and the positivity of the +587G allele were significantly higher in the severe CP group than in the controls ($p = 0.0097$ and 0.0075, respectively). Considerably, the TNFR2(+587G) allele positive subjects exhibited more severe signs of periodontitis than negative subjects.

Ethnic and racial differences in disease-susceptibility gene polymorphisms have been reported. For example, different frequencies of the TNFR2(+587T/G) gene polymorphism have been found in Caucasians, African-Americans and Japanese. Positivities of the TNFR2 (+587G) polymorphic allele in healthy subjects were 45% in Caucasians (Barton et al. 2001), 33% in African-Americans (Bridges et al. 2002) and 17% in our Japanese data. The rate of TNFR2(+587G) polymorphic allele was lower in Japanese than Caucasians whereas the rate in African-Americans was between the Caucasians and the Japanese data.

Several studies have indicated the associations between the TNFR2 (+587T/G) gene polymorphism and some chronic inflammatory and autoimmune diseases (Komata et al. 1999, Nishimura et al. 2000, Barton et al. 2001, Morita et al. 2001). Kinane et al. (1999) have suggested that periodontitis could have similarities in causative

genetic factors to chronic inflammatory and autoimmune diseases, including SLE and RA, and it may be useful to examine these diseases associated with periodontitis. If the phenotype for factors, which could be present in the periodontium as well as conventional sites of these diseases plays a role in disease outcome, those same factors may be also important in periodontitis. The TNFR2(+587G) allele has been associated with the susceptibility to SLE in Japanese (allele positivity: 37.0%, 37.1% in SLE patients versus 18.8%, 25.3% in controls, respectively) (Komata et al. 1999, Morita et al. 2001), RA in Caucasians (56.7% in RA patients versus 43.3% in controls) (Barton et al. 2001) and narcolepsy in Japanese (30.2% in narcolepsy patients versus 20.1% in controls) (Hohjoh et al. 2000). In contrast, it has been shown that the +587G allele has nothing to do with SLE in UK and Spanish population (Al-Ansari et al. 2000) as well as RA in Japanese (Shibue et al. 2000). It can be concluded that the TNFR2(+587G) polymorphic allele is involved in susceptibility or severity factors to several chronic inflammatory and autoimmune diseases including periodontitis though ethnic and racial differences remain to be added in the disease-susceptible genotype.

As shown in Table 3, we further clarified the clinical relevance of the TNFR2(+587T/G) gene polymorphism in non-smoking CP patients. The mean measured values of PPD (mm), CAL (mm) and BL (%) were significantly higher in the +587G positive than in the negative subjects ($p = 0.035$, 0.022 and 0.018, respectively), while the other clinical parameters, such as age, gender, and accumulation of plaque were not significantly different. Moreover, we performed a logistic regression analysis to evaluate genotype effects on an increased severity of CP while adjusting for possible confounding factors such as age, gender, and accumulation of plaque. The logistic regression model clearly showed a strong correlation between the TNFR2(+587T/G) genotype and an increased severity of CP ($p = 0.0067$; odds ratio = 3.45). Thus, the TNFR2(+587T/G) gene polymorphism identified here could be a candidate marker of some biologic changes that are sufficiently strong to lead to severe CP in the absence of smoking and without regard on the level of bacterial challenge.

Smoking is known to be one of the important risk factors of periodontitis and the smoking-related risk could obscure the polymorphism-related increase in risk (Kornman et al. 1997). For this reason, we have focused on the TNFR2(+587T/G) gene polymorphism in non-smoking periodontitis patients, indicating a siginificant association of the +587G allele with the severity of CP. Recent studies have suggested evaluating possible interactions of both risk factors, smoking and genotype (McDevitt et al. 2000, Meisel et al. 2002), so it would be interesting to further investigate the interaction between smoking and TNFR2(+587T/G) genotype effects on periodontal disease.

This study has indicated that the TNFR2(+587T/G) gene polymorphism could be a candidate marker of severe periodontitis. On the other hand, periodontitis is a multifactorial disease and it is likely that there are multiple genes that will be found to be associated with disease risk, and the effects of these genes may not be independent of each other (Schenkein 2002). Thereby it is more likely to be explained by multiple gene polymorphisms. However, for the utility of such a gene polymorphism test in the diagnosis of and screening for periodontitis, we should take into account the importance of determining sensitivity and specificity. The TNFR2 (+587T/G) gene polymorphism identified here exhibited weak sensitivity (37.9%) but high specificity (82.6%). Thus, we would like to attempt to further search for such candidate gene polymorphisms (e.g. other cytokines and their receptors) and to simultaneously perform multiple analysis including a variety of genetic and environmental factors in order to reach higher sensitivity.

Concerning functional studies related to the TNFR2(+587T/G) gene polymorphism, Morita et al. (2001) have recently demonstrated significantly increased IL-6 production by the TNFR2 (+587G) when compared with the TNFR2(+587T), using TNFR2-transfected HeLa cells. The cytotoxic activity induced by the TNFR2(+587G) is also increased when compared with that of the TNFR2(+587T). Although the TNFR2(+587T/G) polymorphism is located within cysteine-rich domains, which is important for optimal TNF-α, it does not have any effect on TNF-α binding. In addition, the concentration of soluble TNFR2 in the culture media is not different in TNFR2(+587T) or (+587G)-transfected HeLa cells when standardized by the TNFR2 number in these cells. They have concluded that TNFR2(+587G) transduces the signals of TNF-α more effectively than does TNFR2(+587T), resulting in increased IL-6 production without affecting the affinity of TNF-α for TNFR2. Komata et al. (1999) have also suggested that the 196 Arg substitution (+587G allele) may have a loss of function, resulting in an excessive immune response (e.g. overproduction of inflammatory cytokines) in individuals carrying the TNFR2(+587G) allele. On the other hand, it has been reported that TNFR2 microsatellite polymorphisms in intron 4 are associated with soluble TNFR2 levels in human serum as well as coronary artery diseases and hypertension (Glenn et al. 2000, Benjafield et al. 2001). Also, these polymorphisms and +587(T/G) polymorphism could be in linkage disequilibrium. These observations imply that TNFR2(+587T/G) polymorphism could be expected to be in linkage disequilibrium with any other nearby functional polymorphisms. Taken together, the altered function of the TNF-α receptor side may be involved in the pathogenesis or progression of periodontitis in subjects carrying the TNFR2(+587G) allele.

In conclusion, our study has revealed that the frequency and positivity of the TNFR2(+587G) polymorphic allele are significantly increased in Japanese non-smoking patients with severe chronic periodontitis. This allele positive subjects also exhibited more severe signs of periodontitis than negative subjects. These findings suggest that the TNFR2 (+587G) polymorphic allele could be associated with severe chronic periodontitis in Japanese. However, further population and functional studies are needed to confirm our findings.

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