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ORIGINAL ARTICLE

The Tumor Necrosis Factor-Alpha Gene-857 Single-Nucleotide Polymorphism Associated with Early Implant Failure in Asian Patients

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ABSTRACT

This study examined the association between implant failure and IL-1β (+3954) and TNF-α (–857) gene polymorphisms in Japanese. Forty patients (mean age 64.1 years) were divided into a control group with one or more healthy implants (n = 27) and a test group with one or more implants that failed early (n = 13). Samples were collected from the buccal mucosa using spatula. Total genomic DNA was amplified using phi29 DNA polymerase. Genomic DNA from the buccal mucosa was amplified using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The significance of the differences in the observed frequencies of the allele ratio and genotype distribution in both groups was assessed by Monte Carlo simulations using the program CLUMP. In the patient analysis, no significant differences were found in the allele or genotype distributions of the IL-1β gene between the control and test groups, and of TNF-α. In the implant analysis, no significant differences were noted in the allele or allele T-containing genotype distributions of IL-1β (+3954) between the control and test groups, whereas the allele distribution of TNF-α (–857) differed significantly between the two groups (p = 0.02). In the patient analysis, the patients possessing both genotypes, i.e. the allele T at IL-1β (+3954) and TNF-α (–857), the difference between the control and test groups was close to significance (p = 0.055). In the implant analysis, the patients possessing both genotypes, i.e. allele T at IL-1β (+3954) and TNF-α (–857) the difference between the control and test groups was significant (p = 0.008). This study suggests that possessing both the IL-1β (+3954) and TNF-α (–857) single-nucleotide polymorphisms (SNPs) constitutes a risk factor for early implant failure in Japanese.

Keywords: TNF-α, IL-1β, Polymorphism, Implant failure, Japanese.

INTRODUCTION

Dental implants have been used to treat patients with missing teeth with high success rates,[1,2] since Brånemark et al[3] reported the osseointegration of a titanium surface in direct contact with bone. Nevertheless, implant failure occurs, and such failures can be divided into early failure due to the failure of osseointegration and late failure which occurs after implant loading with functional pressure.[4] Early failure occurs when osseointegration is not achieved due to surgical trauma, unfavorable initial fixation or bacterial infection at the implant site. Late failure occurs when osseointegrated implants are overloaded or bacterial infection induces peri-implant inflammation.[5] However, some implant failure phenomena cannot be explained by these factors.

Peri-implant inflammation is caused by bacterial infection in the peri-implant region. As a result, pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) are produced and activate osteoclasts, promoting bone resorption and inducing matrix metalloproteinase production by fibroblasts.[6] This results in the destruction of hard and soft peri-implant tissues and the loss of peri-implant supporting bone.

The recent analysis of the human genome has revealed the presence of single-nucleotide polymorphisms (SNPs) that affect cytokine production. It has been suggested that these gene polymorphisms determining the production of TNF-α and IL-1β are associated with the development or progression of periodontitis and peri-implant inflammation.

Reported SNPs include those at +3953[7], –511[8], and –31[9] from the transcription initiation site in the IL-1β gene and at –308[10] and –238[11] in the TNF-α gene. These IL-1β and TNF-α gene polymorphisms are among the most frequently noted gene polymorphisms in reports of associations between severe periodontal disease and IL-1β[12] and TNF-α gene polymorphisms.

Several reports have identified associations between peri-implant inflammation and these polymorphisms,

including associations between IL-1β gene polymorphisms and the early resorption of peri-implant bone,[13] and between TNF-α gene polymorphisms and early implant failure.[14] However, the allele distribution of these polymorphisms varies among races. The prevalence of IL-1β (+3953, –511, –31) and TNF-α (–308, –238) polymorphisms is high in Caucasians but low in Asians,[15,16] indicating that findings in different races cannot be applied to Japanese directly.

A recent study of TNF-α gene promoter region of Japanese discovered an SNP at –857 present at high frequency,[17] and its association with severe periodontal disease has been reported.[16] However, few studies have examined the association between implant failure and gene polymorphisms in Japanese.

Therefore, this study examined the association between implant failure and the IL-1β (+3954) and TNF-α (–857) gene polymorphisms in Japanese.

MATERIALS AND METHODS

Subject Selection

The subjects were 40 patients who had Nobel Biocare™ and ITI implants placed at the Department of Oral Reconstruction and Rehabilitation of Kyushu Dental College, and who gave informed consent to participate in this study (Table 1).

The baseline clinical parameters of the subject population are presented in Table 1. The subjects were divided into two groups: A control group with one or more healthy implants (n = 27) and a test group who had early failure of one or more implants (n = 13). An implant was considered to have been lost early when mobility or pain developed before or during functional loading.

The subjects had to be in good general health and were excluded if they were smokers; had human immunodeficiency virus (HIV) infection; had a history of chronic illnesses, such as cardiovascular disease, diabetes, osteoporosis, immune, or bleeding disorders; had undergone radiotherapy or chemotherapy; were currently pregnant or lactating; used orthodontic appliances; had necrotizing ulcerative gingivitis or periodontitis; or had a history of aggressive periodontitis.

DNA Collection and Extraction

Samples were collected from the buccal mucosa using a spatula. The cells were examined using Giemsa staining. After lysing the cells, total genomic DNA was amplified using phi29 DNA polymerase.

Analysis of Genetic Polymorphisms

The IL-1β and TNF-α SNPs were detected using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). PCR was performed using AmpliTaq Gold® DNA polymerase (Perkin-Elmer Cetus, Norwalk, CA, USA) in a 50 ml reaction mixture containing 1.5 mM $MgCl_2$ according to the manufacturer's instructions.

For IL-1β (+3954), the primers used were (sense) 5'CTC AGG TGT CCT CGA AGA AAT CAA A 3' and (antisense) 5'GCT TTT TTG CTG TGA GTC CCG 3' (12) with 1.5 mM $MgCl_2$. The PCR consisted of an initial 10 minutes at 95°C followed by 35 cycles of 30 seconds at 94°C, 60°C, and 74°C. The products were digested with 3 units of TaqI at 65°C for 10 minutes. The C allele gave products of 12, 85, and 97 bp whereas the T allele gave products of 12 and 182 bp.

For TNF-α (–857), the primers used were (sense) 5'CCC CAG TGT GTG GCC ATA TCT TCT T 3' and (antisense) 5'TGG AGG CAA TAG GTT TTG AGG 3' (19) with 1.5 mM $MgCl_2$. The PCR consisted of an initial 10 minutes at 95°C followed by 35 cycles of 30 seconds at 94°C, 60°C, and 74C. The products were digested with 3 units of HincII

Table 1 Clinical characteristics of the study population (n = 40)

	Test group (n = 13)		*Control group (n = 27)*	
	n	*%*	*n*	*%*
Age (years)	65.54 ± 7.42		62.59 ± 8.71	
Gender				
– Male	5	38.5	9	33.3
– Female	8	61.5	18	66.7
Implant region (n = 177)				
	Lost implants (n = 18)		Healthy implants (n = 159)	
	n	%	n	%
Position				
Maxilla	17	92.9	90	54.4
Mandible	1	7.1	69	45.6
Anterior	6	21.4	64	43.7
Posterior	12	78.6	95	64.3

at 58°C for 10 minutes. The C allele gave products of 108 and 25 bp whereas the T allele gave a 133 bp product.

Statistical Analysis

The significance of the differences in the observed frequencies of allele ratio and genotype distribution in both groups was assessed using Monte Carlo simulations using the program CLUMP. $P < 0.05$ was considered significant.

RESULTS

A total of 177 implants were placed in 40 patients (14 males, 26 females; mean age 64.07 years). Implant failure occurred in 13 patients, and 18 implants were lost (See Table 1).

At IL-1β (+3954), allele T was detected at a frequency of 3.7% in the control group and 15.4% in the test group. Allele T-containing genotypes were detected in 7.4 and 23.1% of the control and test groups respectively. At TNF-α (–857), allele T was detected at frequencies of 27.8 and 38.5% in the control and test groups respectively, and allele T-containing genotypes were present in 44.4 and 61.5% respectively.

No significant differences were seen in the allele ($p = 0.08$) or genotype ($p = 0.25$) distribution of the IL-1β gene between the control and test groups, nor were there significant differences in the allele ($p = 0.45$) or genotype ($p = 0.63$) distributions of TNF-α (Table 2).

On analysis based on implant survival, allele T at IL-1β (+3954) was detected at frequencies of 7.9 and 11.1% in the control and test groups, and the allele T-containing genotypes were present in 15.1 and 16.7% respectively. Allele T at TNF-α (–857) was detected in 29.9 and 50% in the control and test groups respectively, and the allele T-containing genotypes were present in 46.5 and 72.2% respectively.

No significant differences were seen in the allele ($p = 0.51$) or allele T-containing genotype ($p = 0.25$) distributions of IL-1β (+3954) between the control and test groups whereas the allele distribution of TNF-α (–857) differed significantly between the two groups ($p = 0.02$, Table 3).

The number of patients with genotypes containing allele T at both IL-1β (+3954) and TNF-α (–857) were compared between the control and test groups; the difference approached statistical significance ($p = 0.055$, Table 4). When the implant survival with patient genotypes containing allele T at both IL-1β (+3954) and TNF-α (–857) was also compared between the control and test groups, a significant difference was found between the two groups ($p = 0.008$, Table 5).

DISCUSSION

The main causes of implant failure are infection, overheating occuring when forming the socket for implant placement, pressure loading too early, overloading after functional loading, and healing disorders.[5,18] However, some causes of implant failure cannot be explained by these factors (21), implying that there is a group at high risk of implant failure caused by individual characteristics, such as hereditary factors.

Although various studies have looked for an association between IL-1β gene polymorphisms and implant failure, none has been found.[19-21] However, Boris et al[22] reported that although there was no association between implant failure and the IL-1β gene polymorphism, the gene polymorphism was strongly correlated with drainage from the peri-implant gingival sulcus, fistula formation, and biological complications accompanied by bone resorption on X-rays in heavy cigarette smokers. Feloutzis et al[23] also found that the peri-implant bone resorption during the maintenance step after prosthesis application was

Table 2 Distribution of IL-1β (+3954) and TNF-α (–857) alleles and gene polymorphisms in the control and test groups (based on patients)

	Test group (n = 13)	*Control group (n = 27)*	*p-value*
IL-1β alleles			
c	22(84.6%)	52 (96.3%)	
t	4(15.4%)	2(3.7%)	0.08
IL-1β genotypes			
c/c	10 (76.9%)	25(92.6%)	
c/t	2(15.4%)	2(7.4%)	
t/t	1(7.7%)	0(0%)	0.51
TNF-α alleles			
c	16(61.5%)	39(72.2%)	
t	10(38.5%)	15(27.8%)	0.45
TNF-α genotypes			
c/c	5(36.5%)	15(55.6%)	
c/t	6(46.2%)	9(33.3%)	
t/t	2(15.3%)	3(11.1%)	0.63

Table 3 Distribution of IL-1β (+3954) and TNF-α (–857) alleles and gene polymorphisms in the control and test groups (based on patients)

	Test group (n = 18)	*Control group (n = 159)*	*p-value*
IL-1β alleles			
c	32(88.9%)	293 (92.1%)	
t	4(11.1%)	25 (7.9%)	0.51
IL-1β genotypes			
c/c	15 (83.3%)	135(84.9%)	
c/t	2(11.1%)	23(14.5%)	
t/t	1(5.6%)	1(0.6%)	0.25
TNF-α alleles			
c	18(50.0%)	223(70.1%)	
t	18(50.0%)	95(29.9%)	0.02
TNF-α genotypes			
c/c	5(27.8%)	85(53.5%)	
c/t	8(44.4%)	53(33.3%)	
t/t	5(27.8%)	21(13.2%)	0.09

Table 4 Distribution of the composite genotype allele T IL-1b (+3954) and TNF-α (–857) in the control and test groups (based on patients)

	Test group n = 13	*Control group n = 27*	*p-value*
Composite genotype	3 (23.1%)	1(3.7%)	
Other genotype	10(76.9%)	26(96.3%)	0.055

Table 5 Distribution of the composite genotype allele T IL-1β (+3954) and TNF-α (–857) in the control and test groups (based on implants)

	Test group n = 18	*Control group n = 159*	*p-value*
Composite genotype	3 (16.7%)	2(1.3%)	
Other genotype	15(83.3%)	157(98.7%)	0.008

significantly higher in heavy cigarette smokers with the IL-1β gene polymorphism. These studies suggest an association between the IL-1β gene polymorphism and peri-implant bone resorption in heavy cigarette smokers. The prevalence of the gene polymorphism at IL-1β (+3954) varies among races. The prevalence of allele T is 15 to 25% in Caucasians, and 25 to 50% of genotypes contain allele T.[12,24] Conversely, the prevalence of the allele T in Asians is only 3 to 10%, and the genotypes account for 5 to 15%.[13,15,25] In our study, the incidence of allele T at IL-1β (+3954) was 6.9%, and 11.1% of the genotypes contained allele T, consistent with previous studies and no correlation with implant failure was seen as reported previously.

Regarding the association between the TNF-α gene polymorphism and implant failure, Campos et al[14] found no significant difference in the allele or genotype distributions of the TNF-α (–308) gene polymorphism between a test group with one or more failed implants and a control group with one or more healthy implants showing no relationship between the TNF-α (–308) gene polymorphism and early implant failure. Cury et al[26] also reported no association between the amount of peri-implant bone resorption 6 to 31 months after implant placement and the TNF-α (–308) gene polymorphism in patients. Subsequently, they reported the absence of an association between implant failure and the TNF-α (–308) gene polymorphism.[27,28] The prevalence of allele A at the TNF-α (–308) gene exceeds 20% in Caucasians,[27,29] but is only 1 to 3% in Japanese.[16] The prevalence of the TNF-α (–857) gene polymorphism is specifically high in Japanese,[17] and associations with rheumatism,[30] type 2 diabetes mellitus,[31] and Crohn's disease[32] have been suggested. In addition, an association with severe periodontitis has been reported.[16] Conversely, in a Brazilian study of the association of implant failure with polymorphisms, such as the TGF-β and MMP-1 and 9 genes,[33] no association was demonstrated.

This is the first study to examine the association between the TNF-α (–857) gene polymorphism and Japanese patients with early implant failure. There was a significant difference in the distribution of allele T at TNF-α (–857) between the implant-based control and test groups (p = 0.02, Table 3), but no significant difference was seen between the patient groups (p = 0.45, Table 2). A single gene polymorphism may not constitute a definitive cause. When patients possessing both genotypes, i.e. allele T at both IL-1β (+3954) and TNF-α (–857) were compared between the control and test groups, the difference approached significance (p = 0.055, Table 4). Furthermore, when the patient genotypes in the implant control and test groups were compared, a significant difference was found between two groups in the presence of allele T at both IL-1β (+3954) and TNF-α (–857) (p = 0.008, Table 5). Consequently, our results suggest that possessing both the IL-1β (+3954) and TNF-α (–857) single-nucleotide polymorphisms (SNPs) is a risk factor for early implant failure in Japanese.

In addition, there are many other polymorphisms in the promoter regions of genes associated with inflammatory cytokine induction. Once these are all elucidated, and a combination of gene polymorphisms clearly associated with implant failure is identified, these findings may be useful for screening before implant treatment and may help to increase the implant success rate in specific ethnic groups.

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