The effect of genetic polymorphisms on treatment duration following premolar extraction

Jiyon Yu

Department of Dentistry
The Graduate School, Yonsei University

The effect of genetic polymorphisms on treatment duration following premolar extraction

Directed by Professor Jung-Yul Cha

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This certifies that the Doctoral Dissertation of Jiyon Yu is approved.

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Thesis Supervisor: Jung-Yul Cha

_____

Yoon Jeong Choi

_____

Sung-Hwan Choi

_____

Han-Sung Jung

_____

Ji Hyun Lee

The Graduate School

Yonsei University

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감사의 글

부족한 저를 관심과 사랑으로 이끌어 주시고, 학위 과정에서 수많은 조언과 지도를 해주신 차정열 지도 교수님께 제일 먼저 감사의 말씀을 드립니다. 그리고 항상 세심하게 신경 써 주시고, 논문이 나오기까지 많은 도움을 주신 이지현 교수님께도 마음 깊이 감사드립니다. 또한 관심과 격려로 논문 심사를 맡아 주신 최윤정 교수님, 최성환 교수님, 정한성 교수님께도 감사드립니다.

교정학에 입문하여 깊이 있는 공부를 할 수 있게 이끌어 주시고 인생의 멘토로서 올바른 길을 보여주시는 플러스원 치과 이주영 원장님께 감사의 마음을 드립니다.

마지막으로 치과의사로서 학업과 병원일, 가정생활을 함께 해 나갈 수 있게 물심양면으로 도와 주시는 양가 부모님들과 가장 든든한 후원자이자 조력자인 남편, 그리고 오랜 기다림 후에 만난 귀한 선물, 하윤이에게 사랑과 감사의 말을 전합니다.

2021 년 12 월

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The effect of genetic polymorphisms on treatment duration following premolar extraction

Jiyon Yu, D.D.S., M.S.D.

Department of Dentistry

The Graduate School of Yonsei University

(Directed by Professor Jung-Yul Cha, D.D.S., M.S.D., Ph.D.)

To elucidate genetic factors affecting orthodontic treatment duration, targeted next-generation sequencing on DNA from the saliva of 117 patients undergoing orthodontic treatment after premolar extraction was performed. The clinical characteristics of patients are summarized, and the association of clinical variables with treatment duration was assessed. Patients whose treatment duration deviated from the average were classified into an extreme long group or an extreme short group. Nine single nucleotide polymorphisms (SNPs) of six genes that significantly differed in the two groups were identified via targeted sequencing. The frequency of the CC genotypes of *WNT3A*, *SPP1* (rs4754, rs9138), and

TNFSF11, TT genotype of *SPP1* (rs1126616), and GG genotype of *SFRP2* was significantly higher in the extreme long group than in the short group. In the extreme short group, the TC genotype of *SPP1*, AA genotype of *P2RX7*, CT genotype of *TNFSF11*, and AG genotype of *TNFRSF11A* tended to exhibit higher frequency than in the long group. Taken together, genetic polymorphisms related to treatment duration in Korean orthodontic patients undergoing premolar extraction were identified. These findings could lead to further studies predicting the prolongation of the orthodontic treatment duration, and will be of great aid to patients as well as orthodontists.

Keywords: orthodontic treatment duration, genetic polymorphism, targeted next-generation sequencing, *WNT3A*, *SPP1*, *SFRP2*, *P2RX7*, *TNFSF11*, *TNFRSF11A*

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Jiyon Yu, D.D.S., M.S.D.

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I. Introduction

Treatment duration is one of the greatest concerns for orthodontists and their patients. Patients are often eager to know the treatment duration and how long they will require to use braces. Orthodontists sometimes change original treatment plans due to prolonged treatment durations. Further, if difficulties arise that complicate regular visits, such as emigration, studying abroad, military enlistment, or long-distance moving, the original

treatment plans are often changed for short-term treatment methods. Timely completion of orthodontic treatment allows for accurate prediction of costs throughout the treatment period and improves treatment efficiency. Further, it can increase patient satisfaction and improve their quality of life. In contrast, prolonged treatment durations 'burn-out' the patients and reduce their cooperation, which inevitably worsens treatment outcome (Pachêco-Pereira et al., 2015). Therefore, the prediction of the orthodontic treatment period is of great importance for establishing the treatment plan and improving patient management for a better outcome.

Several studies have investigated variables that could influence treatment duration. These factors include age (Beckwith et al., 1999; Dyer et al., 1991), sex (Beckwith et al., 1999; Haralabakis and Tsiliagkou, 2004; Skidmore et al., 2006), orthodontic appliances used (Beckwith et al., 1999), magnitude of the orthodontic force (Ren et al., 2003), molar relation (Skidmore et al., 2006), the severity of malocclusion (Mavreas and Athanasiou, 2008; Vu et al., 2008), tooth extractions (Beckwith et al., 1999; Haralabakis and Tsiliagkou, 2004; Mavreas and Athanasiou, 2008; Skidmore et al., 2006), patient cooperation (e.g. missed appointments, breakage of appliances, wearing of elastics), and poor oral hygiene (Fisher et al., 2010). However, these reports had conflicting results with regard to the same variables. For example, some studies suggested that orthodontic treatment duration could be influenced by patient cooperation (Beckwith et al., 1999; Melo et al., 2013; Skidmore et al., 2006), while others reported a low correlation between duration and cooperation (Grewe and Hermanson, 1973). Further, while some authors reported that initial severity

of malocclusion, types of orthodontic appliances, and orthodontic force may affect treatment duration (Haralabakis and Tsiliagkou, 2004; Mavreas and Athanasiou, 2008; Skidmore et al., 2006; Vu et al., 2008), others rejected these claims (Dyer et al., 1991; Ren et al., 2003).

In addition, individual differences in relevant biological characteristics such as alveolar bone density, shape, and bone turnover rate may influence orthodontic tooth movement and thus affect the treatment period (Chugh et al., 2013). Alveolar bone metabolism is closely related to the speed of orthodontic tooth movement, resulting in different bone turnover rates under orthodontic forces based on patient-specific traits (Leung et al., 2009; Shoji-Matsunaga et al., 2017). In an experimental study on beagle dogs, the group whose bone density was increased by promoting bone formation exhibited significantly reduced tooth movement speed compared to the control group, indicating that the quality of alveolar bone affected orthodontic treatment duration (Machibya et al., 2018).

These individual differences in physiological characteristics stem from respective genetic differences attributed to gene polymorphisms that lead to varied gene expression levels between individuals. In other words, genetic polymorphisms may contribute to differences in orthodontic tooth movement treatment duration. Multiple genetic polymorphisms have been related to orthodontic treatment duration, with studies reporting single nucleotide polymorphisms (SNPs) that affect tooth movement. It was reported that the polymorphism of *IL-1*, which encodes an inflammatory cytokine, initially discovered around alveolar bone, affects the speed of tooth movement (Iwasaki et al., 2009; Iwasaki

et al., 2006). In addition, genetic polymorphisms of *RANK*, *RANKL*, *OPG*, *COX2*, and *IL-6* have been reported to influence their expression levels in periodontal ligament fibroblasts, thereby affecting orthodontic tooth movement (Küchler et al., 2019). Furthermore, an SNP of the *SPP1* gene, encoding osteopontin (OPN), was reported to alter bone metabolism in tissues around teeth, subsequently affecting tooth movement and increasing root resorption (Singh et al., 2018).

Despite the importance of genetic factors affecting orthodontic treatment duration, studies on specific genes and polymorphisms that influence tooth movement speed and treatment duration are lacking. In the current study, we hypothesized that genetic polymorphisms affect orthodontic treatment duration due to the resulting individual differences in physiological traits that influence tooth movement. In particular, these physiological differences are to some extent determined by genetic polymorphisms. Thus, we employed bioinformatics to identify genes that influence orthodontic treatment duration in Korean patients who underwent tooth extraction. In addition, genetic polymorphisms related to treatment duration were identified via targeted gene sequencing.

II. Materials and Methods

1. Definition of orthodontic treatment duration

In this study, the duration of orthodontic treatment was defined as the leveling and anterior retraction period for space closing, as these processes are the most relevant to the duration of orthodontic treatment in clinical practice.

2. Subjects

This retrospective study was approved by the institutional review board of Yonsei University Dental Hospital (number: 2-2016-0023). All subjects were Korean participants and unrelated. All clinical examinations were conducted in accordance with the Declaration of Helsinki, and written consent was obtained on paper with a description of the study prior to enrolling all subjects. Initially, 122 patients who received orthodontic treatment with maxillary premolar extractions at the Department of Orthodontics at Yonsei University Dental Hospital in Seoul, Korea from February 2008 to May 2020 were enrolled. Among them, 117 patients (24 males, 93 females, with an average age of 19.8 years) were finally selected; five patients were excluded owing to complications in the genetic analysis due to contamination, or because the patients could not proceed with the treatment due to personal reasons such as long-term absence, poor collaboration, and frequent breakage of brackets

or appliances.

The selection criteria were as follows: (1) treatment with fixed orthodontic appliances in the permanent dentition, (2) maxillary premolar extracted on both sides and anterior teeth retracted for space closing, (3) panoramic and lateral cephalometric radiographs taken before and after orthodontic treatment, (4) no orthognathic surgery, (5) no systemic diseases affecting tooth movement, bone metabolism, or maxillofacial malformation.

All 117 patients were treated using the straight-wire appliance (SWA) technique, from a 0.016-in nickel-titanium to a 0.019 × 0.025-in stainless steel (G & H Orthodontics, Franklin, Ind.) with conventional brackets of 0.022 slots.

3. Measurement of clinical parameters related to treatment duration

To determine the clinical parameters related to orthodontic treatment duration, 18 clinical variables of the subjects such as age, sex, Angle classification, horizontal anterior retraction, crowding, displacement, overjet, overbite, PAR score, ANB (T1: before treatment, T2: after treatment), FMA (T1, T2), and U1 to SN (T1, T2) were measured using pre-treatment dental models, lateral cephalometric radiographs, and chart review. Lateral cephalometric radiographs were acquired before and after orthodontic treatment by a trained radiographer using Cranex3 + (Soredex, Helsinki, Finland) at the Department of Oral and Maxillofacial Radiology, Yonsei University Dental Hospital. These lateral cephalometric radiographs were traced using V-Ceph™ 5.5 (Osstem, Seoul, South Korea).

Displacement, measured in mm, was defined as the sum of the absolute values of crowding and spacing to quantify abnormal tooth position. PAR score was obtained from the PAR index to quantify the degree of initial malocclusion (Richmond et al., 1992). Horizontal anterior retraction was defined as the difference between the tip of maxillary anterior teeth parallel to the HRP (horizontal reference plane) before (T1) and after (T2) orthodontic treatment in the lateral cephalometric radiograph, as measured in mm. HRP, proposed by Burstone (Burstone et al., 1978), was the line minus 7° from the Sella–Nasion line, and it was taken as the horizontal reference of orthodontic tooth movement in the study (Figure 1).

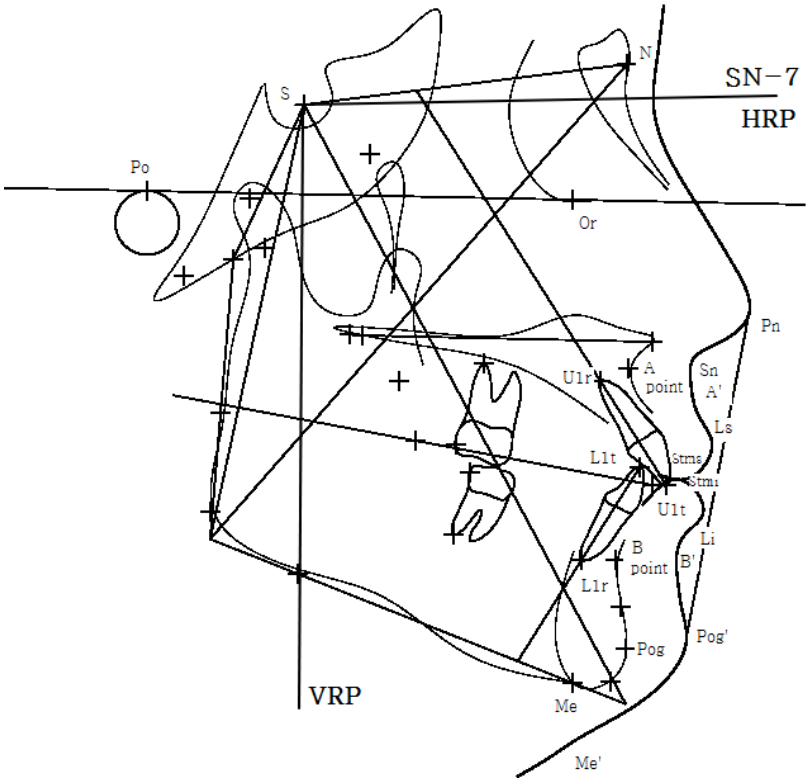

Figure 1. **Reference lines and points for cephalometric analysis.**

Horizontal reference plane (HRP) is line minus 7° from the Sella–Nasion line. Individual landmarks are as follows: sella (S), nasion (N), A point (A), B point (B), pogonion (Pog), menton (Me), root apex of the maxillary central incisor (U1r), tip of the maxillary central incisor (U1t), tip of the mandibular central incisor (L1t), root apex of the mandibular central Incisor (L1r), pronasale (Pn), subnasale (Sn), soft tissue A point (A'), labrale superioris (Ls), stomion superioris (Stms), stomion inferioris (Stmi), labrale inferioris (Li), soft tissue B point (B'), soft tissue pogonion (Pog'), soft tissue menton (Me')

4. Reliability of the measurement method

Ten patients were randomly selected to check for errors in radiographic measurement, and twenty lateral cephalometric radiographs were acquired from them. Each radiograph was examined by the same operator after a 2-week interval to determine reliability of the measurement. The intraclass correlation coefficient (ICC) between the two examinations was over 0.9, indicating considerable consistency. The difference between the first and second assessments was not significant.

5. Genetic analysis

Genomic DNA was isolated from saliva using the Oragene DNA self-collection kit (Genotek, Ottawa, Ontario, Canada) and extracted with the prepIT-L2P DNA extraction kit (DNA Genotek, Ottawa, Ontario, Canada) according to the manufacturer's recommendations. DNA quantification was performed using Qubit.

For targeted next-generation sequencing analysis, previously reported genes and their SNPs related to tooth movement or bone metabolism were screened after a literature review (Table 1). Cytokine genes that induce the inflammatory response around periodontal ligament tissues were selected as genes related to tooth movement. Genes involved in Wnt signaling and the RANK/RANKL pathway were also selected owing to their influence on orthodontic tooth movement and regulation of alveolar bone formation or resorption. Genes included only the coding sequence (CDS) region. For SNPs outside the CDS region,

additional target probes were designed. Hybridization capture-based next-generation sequencing was employed, and sequencing data were obtained using a genome analysis tool kit. Sequencing reads obtained from Illumina NextSeq 500 platforms were further analyzed using BWA-MEM, Picard (v1.115), and Samtools (v1.1), and GATK (v4.0.4.0) was used to call single nucleotide variants.

Table 1. List of targeted sequencing regions

Genes	Additional regions
ALPL	
CASP1	rs530537 (Intron)
CASP5	rs554344 (2KB Upstream)
IL-17A	rs2275913 (2KB Upstream)
IL1A	rs1800587 (5'-UTR)
IL1B	
IL1RN	
IL-6	rs1800796 (Intron)
IL-8	
IRAK1	
LRP1	
LRP5	
LRP6	
DKK1	
DKK2	
DKK3	
FRZB	
FZD7	
SFRP1	rs16890444 (Intron)
SFRP2	rs3242 (3' UTR)
SFRP4	
SFRP5	
WIF1	
WISP3	
WNT10B	
WNT3A	rs4653533 (Intron), rs752107 (Intron)
WNT7B	
SOST	rs1230399 (Upstream), rs851054 (2KB Upstream), rs851056 (2KB Upstream)
SPP1	rs11730582 (2KB Upstream), rs9138 (3'UTR region), rs1126616, rs4754
TNF	rs1800629 (2KB Upstream)
TNFRSF11A	rs12455775 (Intron), rs12956925 (Intron), rs12959396 (Intron), rs12970081 (Intron), rs17069845 (Intron), rs17069898 (Intron), rs17069902 (Intron), rs17069904 (Intron), rs17720953 (Intron), rs3826620 (Intron), rs4426449 (Intron), rs4485469 (Intron), rs4500848 (Intron), rs4524034 (Intron), rs4941125 (Intron), rs4941129 (Intron), rs6567272 (Intron), rs7233197 (Intron), rs7236060 (Intron), rs7237982 (Intron), rs7239667 (Intron), rs8083511 (Intron), rs8086340 (Intron), rs8089829 (Intron), rs8099222 (Intron), rs9951012 (Intron)
TNFRSF11B	rs3102735 (2KB Upstream), rs1032128 (Intron), rs11573856 (Intron), rs11573884 (Intron), rs11573901 (Intron), rs11573938 (Intron), rs1485289 (Intron), rs2875845 (Intron), rs3102724 (Intron), rs3102728 (Intron), rs3134057 (Intron), rs3134060 (Intron), rs7010267 (Intron)
TNFSF11	rs1038434 (Intron), rs12585229 (Intron), rs3742257 (Intron), rs931273 (Intron)
VDR	
P2RX7	

6. Statistical Analysis

Statistical analyses were performed using R 3.5.2 (R Foundation) and GraphPad Prism software (GraphPad Inc., La Jolla, CA, USA). Univariate and multivariate linear regression were performed to analyze the correlation between parameters and orthodontic treatment duration. Differences in clinical parameters between extreme long group (showing long treatment duration) and extreme short group (showing short treatment duration) were analyzed using the $\chi 2$ test or *t*-test, as appropriate. The dominant, codominant, and recessive model was used in genetic association analysis. Genotype frequencies of each SNP were tested using the Hardy–Weinberg equilibrium. All P-values were based on two-sided comparisons, and $P < 0.05$ was considered statistically significant. The degree of linkage disequilibrium (LD) of *SPP1* and *TNFSF11* SNPs was examined using Haploview 4.2 software.

III. Results

1. Correlation between clinical parameters and orthodontic treatment duration

Among the clinical parameters of patients, horizontal anterior retraction was positively correlated (Beta=0.1026, *P* value=0.0014) with treatment duration, while crowding and displacement exhibited a negative correlation (Beta= -0.1241, *P* value=0.0029; Beta= -0.1104, *P* value=0.0041, respectively). Age, sex, Angle classification, overjet, overbite, A point–nasion–B point (ANB), peer assessment rating (PAR) score, Frankfurt mandibular plane angle (FMA), and U1 to SN did not show any significant association. Multivariate linear regression was performed after adding all clinical variables as covariates and the analysis indicated horizontal anterior retraction as the variable most strongly correlated with orthodontic treatment duration (Table 2).

Table 2. Clinical parameters associated with orthodontic treatment duration

Clinical parameters	Univariate analysis		Multivariate analysis	
	OR (95%CI)	***P* value**	**OR (95%CI)**	***P* value**
Age	0.1090 (-0.0358 - 0.2537)	0.1387	0.1349 (-0.0916-0.3614)	0.2455
Gender	0.0004 (-0.0090 - 0.0099)	0.928	-1.7935 (-5.4371-1.8501)	0.3367
Angle Classification	0.0008 (-0.0150 - 0.0165)	0.9247	0.6038 (-1.4962-2.7038)	0.5742
Horizontal anterior retraction	0.1026 (0.0406 - 0.1646)	**0.0014**	0.5889 (0.0102-1.1676)	**0.0485**
Crowding (mm)	-0.1241 (-0.2049 - -0.0433)	**0.0029**	-0.3441 (-1.4399-0.7517)	0.5394
Displacement (mm)	-0.1104 (-0.1852 - -0.0357)	**0.0041**	-0.0299 (-1.2154-1.1556)	0.9607
Overjet (mm)	-0.0217 (-0.0749 - 0.0316)	0.4215	-0.6036 (-1.2411-0.034)	0.0662
Overbite (mm)	0.0244 (-0.0236 - 0.0724)	0.3158	0.2557 (-0.4163-0.9277)	0.4573
ANB(T1)	0.0120 (-0.0445 - 0.0685)	0.6751	-0.188 (-0.8001-0.4242)	0.5485
ANB(T2)	0.0078 (-0.0500 - 0.0657)	0.7887	-0.1933 (-0.7852-0.3986)	0.5235
PAR (unweighted)	-0.0565 (-0.1181 - 0.0052)	0.0725	0.2679 (-0.5177-1.0534)	0.5053
PAR (weighted)	-0.1054 (-0.3368 - 0.1259)	0.3687	0.0796 (-0.0865-0.2457)	0.3497
FMA(T1)	-0.0708 (-0.1985 - 0.057)	0.2748	-0.0959 (-0.3539-0.1621)	0.4679
FMA(T2)	-0.1105 (-0.3126 - 0.0915)	0.2809	-0.0649 (-0.226-0.0963)	0.4316
U1 to SN(T1)	0.0497 (-0.1146 - 0.2139)	0.5504	-0.0329 (-0.2404-0.1746)	0.7565
U1 to SN(T2)	-0.0072 (-0.15 - 0.1356)	0.9207	0.0899 (-0.1448-0.3247)	0.4543

Simple (univariate) and multivariate linear regression analysis was used.

Bold values denote statistical significance at the P value < 0.05 level.

CI, Confidence interval

2. Classification of extreme phenotype groups

The subjects were classified based on treatment duration. After creating a correlation plot between horizontal anterior retraction and orthodontic treatment duration, values outside the standard residual ±1 were set as the extreme groups. Values above the standard residual (+1) were classified into the 'extreme long group' with a treatment duration longer than the average, and values below the standard residual (-1) were classified into 'extreme short group' with a treatment duration shorter than the average. The number of patients in each group was 18.

3. Clinical characteristics of the subjects

The clinical characteristics of the subjects are shown in Table 3. There was no significant difference between groups with regard to age, gender, Angle classification, crowding, displacement, overjet, overbite, ANB, FMA, U1 to SN, as well as PAR score. As expected, treatment duration was significantly different between groups, as they had been classified based on orthodontic treatment duration (Table 3).

Table 3. The clinical characteristics of patients

Clinical variables	All Patients (n=117)	Extreme Short group (n=18)	Extreme Long group (n=18)	P value (Short vs Long)
Age (years), median (range)	19.8(12-46.9)	18.4(12-34.3)	19.5(12-26.7)	0.558
Gender, n (%)				
Female	93(79.5)	15(83.3)	13(72.2)	0.423
Male	24(20.5)	3(16.7)	5(27.8)	
Angle Classification, n (%)				
Class I	44(37.6)	7(38.9)	7(38.9)	
Class II	57(48.7)	10(55.6)	5(27.8)	0.073
Class III	16(13.7)	1(5.5)	6(33.3)	
Horizontal anterior retraction, mean ± SD	4.45(2.79)	4.07(2.82)	3.99(2.77)	0.934
EARR, mean ± SD	2.86(2.38)	2.50(2.29)	3.2(2.78)	0.413
Crowding (mm), mean ± SD	3.72(3.61)	5.14(3.45)	4.16(3.88)	0.424
Displacement (mm),	3.97(3.33)	5.14(3.45)	4.84(2.91)	0.780
Overjet (mm)	3.26(2.29)	3.36(2.01)	2.35(2.11)	0.150
Overbite (mm)	1.57(2.07)	053(2.72)	1.36(1.79)	0.285
ANB(T1)	4.01(2.43)	4.57(2.05)	3.43(3.29)	0.220
ANB(T2)	3.66(2.49)	4.25(1.72)	3.08(3.49)	0.212
FMA(T1)	29.90(5.52)	31.17(6.06)	28.66(6.70)	0.246
FMA(T2)	30.24(8.73)	30.21(5.60)	28.61(6.58)	0.436
U1 to SN(T1)	107.16(7.07)	106.08(8.05)	106.94(9.52)	0.771
U1 to SN(T2)	101.00(6.13)	100.41(6.13)	102.13(6.98)	0.437
PAR (unweighted)	5.22(2.69)	6.00(2.47)	6.17(3.31)	0.865
PAR (weighted)	15.75(9.97)	17.50(9.95)	18.67(12.42)	0.758
Treatment duration (Lv.+retraction)	26.59(7.94)	15.67(4.78)	39.06(5.06)	**<0.001**

* x2 test or t test were used where appropriate.

Bold values denote statistical significance at the $P < 0.05$ level.

4. Association between SNPs and orthodontic treatment duration

Targeted capture sequencing identified 142 variants with a minor allele frequency of >3% in the candidate regions. No common variants were significantly associated with orthodontic treatment duration after Bonferroni correction for multiple testing (cut-off *P*-value = 0.05/142 = 3.52×10^{-4}). Only nine SNPs showed nominal significance ($P < 0.05$) in frequency between the extreme short and long groups (Table 4).

Table 4. SNPs significantly associated with orthodontic treatment duration (leveling and retraction period) in targeted sequencing study

Gene (rs no.)	*Base change (AA change)	Geno-type	Group. No. (%)		**P value [OR (95% CI)]				
			Short	Long	Dominant	Recessive	Codominant		Allele
							Hetero	*Homo*	
***WNT3A* (*rs752107*)**	c.*185T>C	T/T	2(11.1)	0(0)	0.858 [7692.2 (0-2.957)]	**0.027** [10.076 (1.3-78.375)]	0.829 [573.38 (0-6.5082)]	0.777 [4175 (0-4.6521)]	**0.016** [6.529 (1.424-29.93)]
		T/C	6(33.3)	3(16.7)					
		C/C	10(55.6)	15(83.3)					
***SPP1* (*rs4754*)**	c.321T>C (p.D107D)	T/T	1(5.6)	1(5.6)	0.959 [0.927 (0.051-16.71)]	**0.018** [8.935 (1.455-54.88)]	0.45 [0.305 (0.014-6.643)]	0.45 [3.316 (0.148-74.47)]	0.069 [2.902 (0.919-9.17)]
		T/C	11(61.1)	5(27.8)					
		C/C	6(33.3)	12(66.7)					
***SPP1* (*rs1126616*)**	c.789C>T (p.A263A)	C/C	1(5.6)	1(5.6)	0.959 [0.927 (0.051-16.71)]	**0.018** [8.935 (1.455-54.88)]	0.45 [0.305 (0.014-6.643)]	0.45 [3.316 (0.148-74.47)]	0.069 [2.902 (0.919-9.17)]
		C/T	11(61.1)	5(27.8)					
		T/T	6(33.3)	12(66.7)					
***SPP1* (*rs9138*)**	c.*294A>C	A/A	1(5.6)	1(5.6)	0.959 [0.927 (0.051-16.71)]	**0.018** [8.935 (1.455-54.88)]	0.45 [0.305 (0.014-6.643)]	0.45 [3.316 (0.148-74.47)]	0.069 [2.902 (0.919-9.17)]
		A/C	11(61.1)	5(27.8)					
		C/C	6(33.3)	12(66.7)					
***SFRP2* (*rs3810765*)**	c.502+6C>T	G/G	6(33.3)	11(61.1)	0.085 [0.287 (0.07-1.187)]	0.699 [0 (0-2.968)]	0.654 [0.698 (0.146-3.35)]	0.606 [0 (0-11428)]	**0.007** [0.212 (0.068-0.659)]
		G/A	7(38.9)	7(38.9)					
		A/A	5(27.8)	0(0)					
***P2RX7* (*rs3751143*)**	c.1487A>C (p.Q496A)	A/A	14(77.8)	8(44.4)	0.038 [4.968 (1.094-22.56)]	**0.038** [4.968 (1.295-78.38)]	**0.038** [4.968 (1.094-22.56)]	-	0.07 [3.309 (0.906-12.09)]
		A/C	4(22.2)	10(55.6)					
		C/C	0(0)	0(0)					
***TNFSF11* (*rs12585229*)**	c.388-3222C>T	C/C	4(22.2)	11(61.1)	**0.023** [0.17 (0.037-0.781)]	0.516 [0.513 (0.069-3.837)]	**0.028** [0.162 (0.032-0.819)]	0.153 [0.197 (0.021-1.829)]	**0.046** [0.35 (0.125-0.983)]
		C/T	11(61.1)	5(27.8)					
		T/T	3(16.7)	2(11.1)					

Table 4. SNPs significantly associated with orthodontic treatment duration (leveling and retraction period) in targeted sequencing study (continued)

TNFSF11 (rs931273)	c.533-2050C>T	C/C C/T T/T	4(22.2) 11(61.1) 3(16.7)	11(61.1) 5(27.8) 2(11.1)	**0.013** [0.145 (0.032-0.66)]	0.516 [0.513 (0.069-3.837)]	**0.015** [0.127 (0.024-0.667)]	0.147 [0.2 (0.023-1.761)]	**0.029** [0.311 (0.109-0.886)]
TNFRSF11A (rs4524034)	c.616+726 A>G	A/A A/G G/G	2(11.1) 13(72.2) 3(16.7)	7(38.9) 6(33.3) 5(27.8)	0.053 [0.167 (0.027-1.023)]	0.552 [1.687 (0.3-9.48)]	**0.032** [0.128 (0.02-0.834)]	0.405 [0.382 (0.04-3.682)]	0.357 [0.632 (0.238-1.676)]

CI, Confidence interval; OR, odds ratio.

P value was adjusted for gender and age at the beginning of treatment. Bold values denote statistical significance at the $P < 0.05$ level.

*Nucleotide location number was assigned according to the *WNT3A* (Transcript ID: NM_033131.3), *SPP1* (Transcript ID: NM_001251830.1), *SFRP2* (Transcript ID: NM_003013.2), *P2RX7* (Transcript ID: NM_002562.5), *TNFSF11* (Transcript ID: NM_003701.3) and *TNSFRSF11A* (Transcript ID: NM_003839.3) mRNA sequence. Minor allele sequence is underlined in each position

**Dominant and recessive model of genetic association study was based on alternative allele.

In the extreme long group, the frequency of the CC genotypes of *WNT3A*, *SPP1* (rs4754, rs9138), and *TNFSF11* (rs12585229, rs931273), the TT genotype of *SPP1* (rs1126616), as well as the GG genotype of *SFRP2*, was significantly higher than that in the short group. In contrast, the frequency of the TC genotype of *SPP1* (rs4754 and rs1126616), the AC genotype of *SPP1* (rs9138), the AA genotype of *P2RX7*, and the CT genotype of *TNFSF11* was higher in the extreme short group when compared with that in the extreme long group. Furthermore, the heterozygote of *TNFRSF11A* (AG genotype) showed overdominance in the extreme short group.

Three SNPs of *SPP1* (rs4754, rs1126616, rs9138) were linked together and rs9138, as an effective SNP, remained statistically significant after haplotype analysis was performed. The SNPs of *TNFSF11* (rs12585229, rs931273) were also linked together. Rs752107 of *WNT3A* and rs9138 of *SPP1* were identified as 3'-UTR SNPs. The rs3751143 SNP of *P2RX7* was a missense variant, while the SNPs of *TNFSF11* (rs12585229, rs931273) and *TNFSF11A* (rs4524034) were intron variants. The rs3810765 SNP of *SFRP2* was located in a splicing region involved in transcription. Remaining SNPs were synonymous variants encoding the same amino acid sequences.

In all subjects, genetic association with orthodontic treatment duration for significant SNPs of extreme phenotype sampling study was showed on Table 5. The SNPs significantly related to the treatment duration are represented using scatter plots.

Table 5. Genetic association with orthodontic treatment duration in all subjects for significant SNPs of extreme phenotype sampling study.

Gene	SNP	Ref > Alt	Model	Beta (95%CI)	P value
WNT3A	rs752107	T>C	Additive	0.01165 (-0.002322 - 0.02562)	0.1014
			Dominant	0.004399 (-0.001086 - 0.009883)	0.1149
			Recessive	0.007249 (-0.003996 - 0.01849)	0.2042
SPP1	rs4754	T>C	Additive	0.01303 (-0.002230 - 0.02828)	0.0935
			Dominant	-0.0004809 (-0.007303 - 0.006341)	0.8892
			Recessive	0.01351 (0.002163 - 0.02485)	**0.02**
SPP1	rs1126616	C>T	Additive	0.01303 (-0.002230 - 0.02828)	0.0935
			Dominant	-0.0004809 (-0.007303 - 0.006341)	0.8892
			Recessive	0.01351 (0.002163 - 0.02485)	**0.02**
SPP1	rs9138	A>C	Additive	0.01113 (-0.005220 - 0.02748)	0.1802
			Dominant	-0.001527 (-0.009337 - 0.006283)	0.6993
			Recessive	0.01265 (0.001224 - 0.02409)	**0.0303**
SFRP2	rs3810765	C>T	Additive	-0.01125 (-0.02744 - 0.004948)	0.1716
			Dominant	-0.006048 (-0.01686 - 0.004765)	0.2702
			Recessive	-0.005199 (-0.01428 - 0.003885)	0.2593
P2RX7	rs3751143	A>C	Additive	0.003763 (-0.01098 - 0.01851)	0.6141
			Dominant	0.005174 (-0.006475 - 0.01682)	0.3808
			Recessive	-0.001411 (-0.007635 - 0.004813)	0.6542
TNFSF11	rs12585229	C>T	Additive	-0.01369 (-0.02994 - 0.002550)	0.0977
			Dominant	-0.01139 (-0.02241 - -0.0003799)	**0.0427**
			Recessive	-0.002299 (-0.01109 - 0.006491)	0.6054
TNFSF11	rs931273	C>T	Additive	-0.01572 (-0.03208 - 0.0006327)	0.0594
			Dominant	-0.01342 (-0.02447 - -0.002375)	**0.0177**
			Recessive	-0.002299 (-0.01109 - 0.006491)	0.6054
TNFRSF11A	rs4524034	A>G	Additive	-0.006944 (-0.02356 - 0.009671)	0.4095
			Dominant	-0.01036 (-0.01989 - -0.0008305)	**0.0334**
			Recessive	0.003416 (-0.007180 - 0.01401)	0.5244

Simple linear regression analysis was used.

Bold values denote statistical significance at the $P < 0.05$ level.

CI, Confidence interval

The CC type of *SPP1* (rs4754, rs9138) and the TT type of *SPP1* (rs1126616) were associated with longer orthodontic treatment duration when compared to other types (Figure 2). The AA type of *TNFRSF11A* (rs4524034) and the CC type of *TNFSF11* (rs931273) were associated with an increase in treatment duration (Figure 3). Other SNPs did not exhibit any significant difference in orthodontic treatment duration based on genotypes in all subjects ($P > 0.05$).

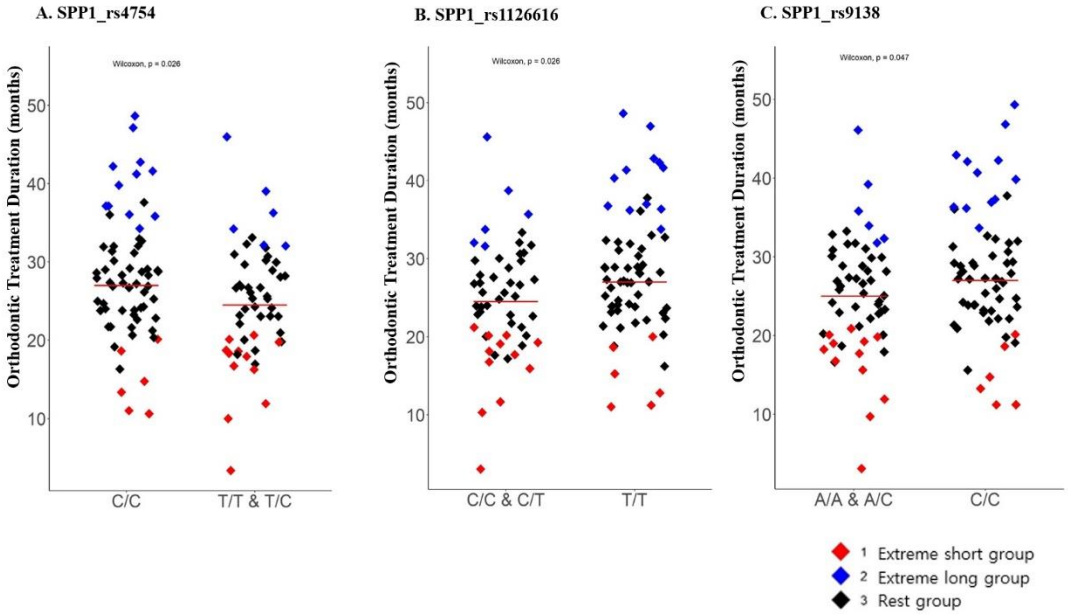

Figure 2. Orthodontic treatment duration according to genotypes in SNPs of *SPP1*

A. *SPP1* (rs4754), B. *SPP1* (rs1126616), C. *SPP1* (rs9138), A scatter plot illustrating the orthodontic treatment duration in each genotype. The y-axis represents the orthodontic treatment duration values (months) and horizontal bars in the scatter plots represent mean values. Statistical analysis was performed with the Wilcoxon test. *P<0.05.

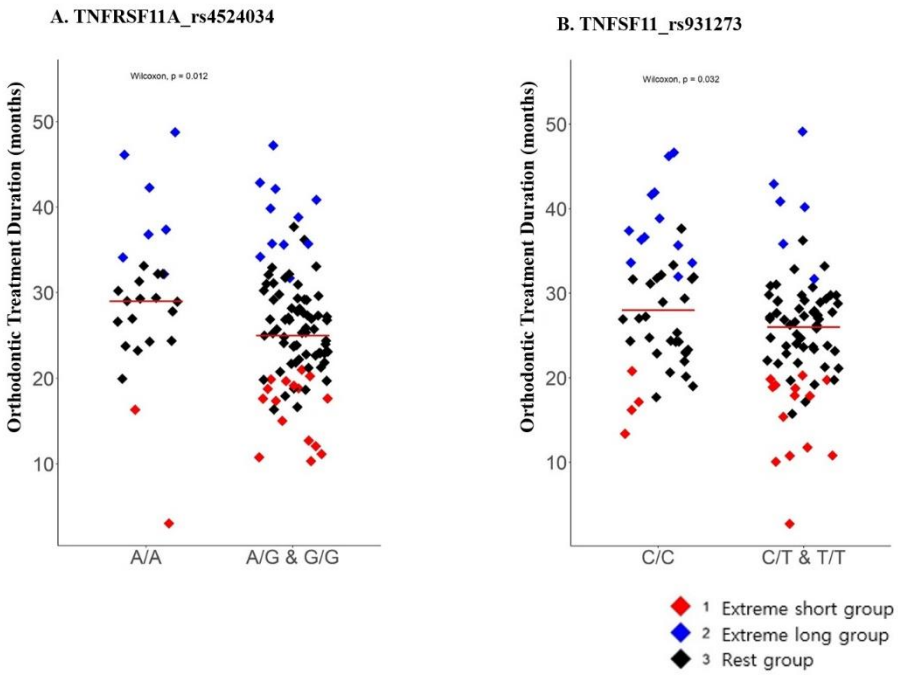

Figure 3. Orthodontic treatment duration according to genotypes in SNPs of *TNFRSF11A* and *TNFSF11*

A. TNFRSF11A (rs4524034), B. TNFSF11 (rs931273)**,** A scatter plot illustrating the orthodontic treatment duration in each genotype. The y-axis represents the orthodontic treatment duration values (months) and horizontal bars in the scatter plots represent mean values. Statistical analysis was performed with the Wilcoxon test. *P<0.05.

The SNPs discovered above and the clinical variables related to the orthodontic treatment duration in the univariate analysis were added as parameters for multivariate analysis to confirm the effect of clinical variables and genetic polymorphisms on the treatment duration. As a result, it was found that rs9138 of *SPP1* and rs4524034 of *TNFRSF11A* affect the orthodontic treatment duration (Table 6). For more details, genetic information of significant SNPs in extreme phenotype sampling study and allele frequency in all subjects were described in Table 7.

Table 6. Predictive ability of SNPs and other clinical parameters for orthodontic treatment duration

Parameters	Beta (95%CI)	P value
Horizontal anterior retraction	0.4612 (-0.1171 - 1.039)	0.1169
Crowding	-0.1903 (-1.294 - 0.9137)	0.7333
Displacement	-0.135 (-1.308 - 1.038)	0.82
Presence of CC Genotype for rs9138	2.918 (0.1756 - 5.661)	**0.0372**
Presence of CC Genotype for rs931273	2.412 (-0.4825 - 5.307)	0.1015
Presence of AA Genotype for rs4524034	3.445 (0.09105 - 6.799)	**0.0442**

The rs9138 of *SPP1* and rs931273 of *TNFSF11* were chosen in multivariate regression analysis for clinical and genetic variable, since three SNPs of *SPP1* (rs4754, rs1126616 and rs9138) and two SNPs of *TNFSF11* (rs12585229 and rs931273) were linked together, respectively.

Bold values denote statistical significance at the $P < 0.05$ level.

CI, Confidence interval

Table 7. Genetic information of significant SNPs in extreme phenotype sampling study and allele frequency in all subjects

Gene	SNP	Type of Variant	Allele Frequency
WNT3A	rs752107	Intron Variant	T=0.22, C=0.78
SPP1	rs4754	Synonymous Variant	T=0.27, C=0.73
SPP1	rs1126616	Synonymous Variant	C=0.27, T=0.73
SPP1	rs9138	3'-UTR Variant	A=0.30, C=0.70
SFRP2	rs3810765	Intron Variant	C=0.56, T=0.44
P2RX7	rs3751143	Missense Variant	A=0.71, C=0.29
TNFSF11	rs12585229	Intron Variant	C=0.59, T=0.41
TNFSF11	rs931273	Intron Variant	C=0.60, T=0.40
TNFRSF11A	rs4524034	Intron Variant	A=0.47, G=0.53

IV. Discussion

In the current work, we explored factors that influence orthodontic treatment duration, with a focus on genetic traits. Univariate linear regression was employed to analyze the clinical parameters of subjects, and horizontal anterior retraction exhibited a positive correlation with treatment duration. In other words, the greater the horizontal retraction of anterior teeth, the longer the treatment duration. In contrast, crowding was negatively associated with orthodontic treatment duration. Crowding was previously reported to influence the increase in leveling duration (Fisher et al., 2010). However, after the completion of leveling, crowding is resolved, and the extraction space decreases, leaving less available space for anterior tooth retraction. According to simple linear regression data on the degree of crowding related to leveling and retraction period (data not shown), the decrease in anterior retraction duration was greater than the increase in leveling duration. Thus, simple linear regression suggested that crowding was associated with a shorter orthodontic treatment duration.

The multivariate linear regression analysis performed thereafter indicated that horizontal anterior retraction was the only clinical variable significantly associated with orthodontic treatment duration. These results are consistent with the lack of consensus between previous studies on clinical factors affecting orthodontic treatment duration. Conflicting results have been reported regarding the effects of age, sex, and Angle classification on

treatment duration (Beckwith et al., 1999; Melo et al., 2013; Skidmore et al., 2006; Vig et al., 1990). There have also been discrepancies on the association of pretreatment ANB, FMA, PAR score, overjet, and overbite with treatment duration (Fink and Smith, 1992; Grewe and Hermanson, 1973; Robb et al., 1998; Vu et al., 2008). Although clinical parameters related to orthodontic treatment duration exhibited a low correlation in the current study, no conclusion can be drawn regarding the relationship of analyzed clinical parameters and treatment duration, as further clinical research is necessary.

Targeted next-generation sequencing revealed nine SNPs in six genes with a significantly different frequency between the extreme groups classified based on horizontal anterior retraction. The frequency of the C allele SNP of *WNT3A* (rs752107) was higher in the extreme long group compared with that in the extreme short group. Wnt signaling is crucial for the growth and maintenance of various tissues, including bone (Rafighdoost et al., 2018). The WNT3A protein is particularly involved in the differentiation of mesenchymal stem cells (MSCs) into osteoblasts (Monroe et al., 2012). It plays an important role in bone remodeling and the maintenance of homeostasis by regulating bone formation through the inhibition of MSC differentiation into the osteoblast lineage under high intracellular WNT3A levels (Qiu et al., 2011).

Rs752107, as a 3'-UTR SNP, may contribute to an increase in bone formation through its effect on microRNA (miRNA) binding affinity. That is, it alters the binding site of miRNA, which normally inhibits *WNT3A* translation by binding the *WNT3A* mRNA transcript harboring the C allele. In contrast, the T allele may compromise miRNA binding and

enhance WNT3A protein translation. Higher WNT3A protein levels would be expected to negatively regulate osteoblast proliferation and differentiation, in turn decreasing bone formation (Díaz-Prado et al., 2012; Qiu et al., 2011). Thus, patients with the CC genotype would be expected to exhibit increased bone formation and higher bone density, consistent with the association observed in the present study (Velázquez-Cruz et al., 2014). Increased bone density may subsequently slow orthodontic tooth movement, prolonging treatment duration (Chugh et al., 2013).

There are few studies on the association between *WNT3A* genetic polymorphisms and orthodontic treatment duration. The current findings suggest the possibility of rs752107 being related to bone remodeling, tooth movement, and thus orthodontic treatment duration. However, further studies are required to elucidate the molecular mechanism through which this SNP regulates bone formation as well as to determine whether rs752107 is in linkage disequilibrium with other functional polymorphisms.

SPP1 encodes OPN, which mediates alveolar bone resorption at the compression side when orthodontic force is loaded. In a recent study, OPN upregulation via ERK- and p38 MAPK-mediated signaling was reported at the tension side during orthodontic tooth movement (Jiang and Tang, 2018). OPN is associated with the shortening of orthodontic treatment duration via the acceleration of orthodontic tooth movement through the regulation of alveolar bone remodeling at both the compression and tension sides in response to orthodontic stress (Singh et al., 2018).

Studies have previously reported the association between genetic polymorphisms of *SPP1* and bone density. The C allele of *SPP1* (rs4754) was suggested to be related to high bone density and low risk of fracture (Zhao et al., 2015), while the T allele of rs4754 was associated with low bone mineral density and a high risk of osteoporosis in women (Chen et al., 2014). In the current study, the frequency of the CC genotype of *SPP1* SNPs (rs4754, rs9138) and the TT genotype of rs1126616 was high in the extreme long group. The C allele of rs4754 is associated with reduced *SPP1* function, resulting in decreased bone resorption and increased bone density. High bone density may slow the speed of orthodontic tooth movement and thus lengthen orthodontic treatment duration (Chugh et al., 2013).

The effects of other SNPs (rs1126616, rs9138) on bone biology and orthodontic tooth movement have not yet been studied, with the exception of a report on the significant relationship of rs9138 with external apical root resorption (EARR) in orthodontic treatment (Iglesias-Linares et al., 2014). As a 3′-UTR SNP, rs9138 can significantly affect miRNA-mediated *SPP1* degradation and its protein levels. Further research is required to assess the association of *SPP1* polymorphisms and orthodontic treatment duration.

We observed a high frequency of the GG genotype of the *SFRP2* SNP rs3810765 in the extreme long group. *SFRP2* encodes secreted frizzled-related protein-2 (sFRP-2), one of the sFRP family proteins that bind directly to Wnt proteins and appear to antagonize their effects by sequestering them from their receptor. However, the role of sFRP-2 in teeth, bone, and supporting tissues remains elusive (Cho et al., 2008). sFRP-2 was reported to enhance the inflammatory response and inhibit bone formation (Sathi et al., 2009); however, another

study showed that SFRP2 activates the osteogenic differentiation of apical papilla stem cells, increasing bone formation (Jin et al., 2017). Although there is no research on the effect of rs3810765 in tooth and bone tissues, as a splice region SNP, rs381076 is expected to affect mRNA splicing, thereby altering RNA stability, and influencing *SFRP2* gene expression. Further molecular studies are necessary to determine how the G allele would contribute to longer orthodontic treatment duration.

The purinergic P2X7 receptor (P2RX7) is a member of the P2X subfamily of purinergic receptors activated by extracellular ATP. *P2RX7* expression has been reported in both osteoclasts and osteoblasts where it regulates bone formation and resorption. Further, P2RX7 was demonstrated to be involved in mechanically-induced signaling between osteoblasts and osteoclasts (Li et al., 2005). Loss of P2RX7 function leads to increased osteoclast numbers and decreased trabecular bone mass (Ke et al., 2003). In *P2RX7* KO mice, alveolar bone formation was not significantly affected, but the susceptibility to external apical root resorption was increased with the application of orthodontic force (Viecilli et al., 2009).

As a missense variant, *P2RX7* SNP (rs3751143) is related to various diseases of bones, teeth, and periodontal tissues. The C allele of rs3751143 might contribute to low bone density and a high risk of osteoporosis in postmenopausal women (Xu et al., 2017). In the current study, the A allele frequency of rs3751143 was high in the extreme short group, whereas that of the C allele was not significantly different in the extreme long group. Nonetheless, no conclusion can be drawn regarding the association between rs3751143 and

orthodontic treatment duration, as reliable results from a greater number of subjects as well as functional studies are required.

In this study, the frequency of the C allele of *TNFSF11* SNPs (rs12585229, rs931273) was significantly increased in the extreme long group, which might be associated with lower expression of the *TNFSF11* gene. *TNFSF11* encodes RANKL (receptor activator of NF-κB ligand), and *TNFRSF11A* encodes RANK (receptor activator of NF-κB). RANKL binds to its receptor RANK to induce the differentiation of osteoclasts through RANKL/RANK signaling (Giacobbo et al., 2020). During orthodontic tooth movement, the expression of *TNFSF11* is increased in osteocytes of the periodontal ligament where the orthodontic force is applied, and bone resorption occurs due to osteoclast activation via enhanced RANKL levels (Yamaguchi, 2009). Thus, RANKL promotes orthodontic tooth movement by inducing bone resorption in the direction of tooth movement. When a drug formulation that slowly releases RANKL was injected into the mesial and distal areas of rat premolars, followed by the application of orthodontic force, faster tooth movement was observed (Chang et al., 2020).

As the SNPs of *TNFSF11* (rs12585229, rs931273) and of *TNFRSF11A* (rs4524034) are intron variants, they are generally expected to have little effect on gene function. However, even an intron variant can affect gene expression at the transcription or translation level, as indicated by previous studies, wherein these SNPs were shown to be related to external apical root resorption (de Castilhos et al., 2019). While there have been some studies on genetic polymorphisms of *TNFSF11* that affect bone metabolism, no research on *TNFSF11*

SNPs rs12585229 and rs931273 has been published. There is only a report relating the rs4524034 SNP of *TNFRSF11A* to menarche in women (Pan et al., 2012). Nevertheless, the genetic polymorphisms of *TNFSF11* and *TNFRSF11A* are worth investigating in depth, considering their important role in orthodontic tooth movement.

This study has several limitations, including a small sample size, setting of the orthodontic treatment duration that excluded finishing period, and retrospective nature of the study. There were also practical difficulties encountered in the process of selecting patients suitable for the set criteria. In addition, this study did not elucidate the functional mechanism of how the SNPs are related to the treatment duration. Future studies are required to elucidate these mechanisms, validate the effect of the SNPs on the orthodontic treatment duration, and determine their influence in other cohort groups.

V. Conclusion

1. Genetic polymorphisms associated with the orthodontic treatment duration was searched by bioinformatics and 9 SNPs in 6 genes were found.
2. To verify the association of SNPs with the treatment duration, an increase in the number of subjects or additional functional studies are required.

VI. References

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국문요약

한국인 소구치 발치교정 환자에서 교정치료 기간과 관련한 유전적 다형성에 관한 연구

<지도교수 : 차 정 열>

연세대학교 대학원

치의학과

유 지 연

치아교정치료에서 치료기간을 단축시키는 것은 교정의사들 뿐 아니라 환자들에게도 큰 관심사이다. 교정치료 기간에 영향을 미치는 요인들은 성별, 나이, 교합관계, 교정력의 종류, 부정교합의 심도, 환자의 협조도 등 여러 가지가 언급되고 있다. 또한 골밀도의 변화나 치조골의 염증반응 등 치아이동과정에 관련된 환자의

생물학적인 특징도 치료기간에 영향을 미칠 수 있다. 특히 환자 개인의 유전자 변이인 SNPs(single nucleotide polymorphisms)은 환자의 생물학적인 개인차를 야기할 수가 있으며 이는 치료기간의 차이를 나타낼 수 있다. 하지만 교정 치료 영역에서 유전적 다형성 (genetic polymorphism)과 치료기간과의 관련성을 연구한 결과는 아직까지 거의 없다. 이번 연구에서는 치료기간에 영향을 미치는 요인들 중 유전적 요인을 생물정보학적 방법으로 연구하기 위하여 차세대염기서열분석법 (targeted next-generation sequencing, NGS)을 통해 치료기간과 관련된 새로운 유전적 다형성들을 찾아보았다.

연세대학교 치과병원 교정과에서 상악 소구치 발치 교정 치료를 받은 117명의 환자들을 대상으로 타액을 채취하고 targeted sequencing위해 DNA을 추출하였다. Targeted sequencing을 위한 candidate은 기존에 발표된 논문들을 기반으로 치아이동 에 영향을 미치는 유전자 및 골대사와 관련한 유전자들의 SNPs(single nucleotide polymorphisms)로 선정하였다. 대상환자들의 임상적인 특징을 정리하고, 치료기간에 영향을 줄 수 있는 여러 임상변수들의 관련성 검증을 하였을 때, 상악 전치의 수평견인량 (horizontal anterior retraction, mm)만이 유의성을 나타냈다. 이를 바탕으로 치료기간이 평균보다 긴 극단연장군 (extreme long group)과 짧은 극단단축군 (extreme short group)으로 환자군을 분류하였고, 선정한 candidate 유전자들을 targeted sequencing하여 두 그룹에서 어떤 SNPs가 유의하게 나타났는지 비교하였다.

NGS 결과, 두 그룹에서 유의한 빈도로 출현한 9개의 SNPs (6개의 유전자)를 발견하였다. 극단연장군 (extreme long group)에서는 극단단축군 (extreme short group)과 비교하여 *WNT3A* SNP의 CC 유전자형(genotype), *SPP1* SNP (rs4754, rs9138)의 CC 유전자형, *SPP1* SNP (rs1126616)의 TT 유전자형, *SFRP2* 의 GG 유전자형, *TNFSF11*의 CC 유전자형이 유의하게 높았다. 반면, 극간단축군에서는 극단연장군에 비해 *SPP1*의 TC 유전자형, *P2RX7*의 AA 유전자형, *TNFSF11*의 CT 유전자형, *TNFRSF11A*의 AG 유전자형의 빈도가 높은 경향을 보였다. 전체 환자군에서 위 유전자들의 빈도 (genotype frequency)를 분석하였을 때 *SPP1, TNFRSF11A, TNFSF11*의 빈도가 유의하게 나타났으며 이 SNP들과 치료기간과의 상관성을 확인하였다.

이번 연구에서는 소구치 발치 교정 치료를 진행한 한국인 환자에서 교정치료기간과 관련이 있는 유전적 다형성을 새롭게 발견하였다. 이들이 치료기간에 미치는 영향력을 검증하기 위해서는 SNPs의 생물학적인 기전을 밝히기 위한 추가적인 연구들이 필요하다.