ORIGINAL ARTICLE

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IL-1 gene polymorphisms, secretion in gingival crevicular fluid, and speed of human orthodontic tooth movement

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Structured Abstract

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Objectives – To investigate genetic, biologic, and mechanical factors that affect speed of human tooth movement.

Setting and Sample Population – Sixty-six maxillary canines in 33 subjects were translated distally for 84 days.

Material and Methods – Distal compressive stresses of 4, 13, 26, 52, or 78 kPa were applied to maxillary canines via segmental mechanics. Dental casts and gingival crevicular fluid (GCF) samples were collected nine to 10 times/subject over 84 days at 1- to 14-day intervals. Three-dimensional tooth movements were measured using a microscope and each subject's series of dental casts. GCF samples were analyzed for total protein, interleukin-1β (IL-1β), and interleukin-1 receptor antagonist (IL-1RA). Cheek-wipe samples from 18 subjects were typed for IL-1 gene cluster polymorphisms.

Results – Average speeds of distal translation were 0.028 ± 0.012, 0.043 ± 0.019, 0.057 ± 0.024, 0.062 ± 0.015, and 0.067 ± 0.024 mm/day for 4, 13, 26, 52, and 78 kPa, respectively. Most teeth moved showed no lag phase (63/66). Three factors significantly affected speed (p = 0.0391) and provided the best predictive model (R^2 = 0.691): Activity index [AI = experimental (IL-1β/IL-1RA)/control (IL-1β/IL-1RA)], IL-1RA in GCF, and genotype at IL-1B.

Conclusions – Increased AI and decreased IL-1RA in GCF plus having ≥1 copy of allele 2 at IL-1B(+3954) were associated with faster tooth movement in humans.

Key words: genetic polymorphism; human; interleukin-1 receptor antagonist; interleukin-1β; tooth movement

Introduction

Over 100 years of orthodontic research and experience has yielded no conclusive evidence-based information regarding ideal biomechanical prescriptions to optimize speed of human tooth movement. That is, factors that affect bone remodeling and tooth movement, such as applied force or stress magnitude, age-related characteristics, cell biology, and

genetics have yet to be established or quantified. This lack of information is a barrier to improving the efficiency of orthodontic treatment.

Surveys to gather information concerning optimum force magnitudes for tooth movement have been conducted (1, 2). These have revealed a remarkable paucity of experimentally based studies. Uncontrolled force systems acting on teeth were common drawbacks (1). Just 17 animal and 12 human studies were revealed (2), where reasonable criteria for study design were applied. Of the 12 human studies, only four involved controlled tooth movement (3–6), and only one (3) quantified magnitude of stresses (force/area). Needless to say, a meta-analysis was not possible because of insufficient data.

The limited number of experimental studies that compared rates of tooth movement in adolescents vs. adults showed faster rates in younger individuals (7, 8). Studies of age-effects on tooth movement in rodents have shown generally higher rates in younger animals (9–13). However, most of these studies were of short duration and the stress magnitudes used were relatively high. In addition, the physiology of rodents in terms of their teeth and aging is very different from humans.

Variability in rate of tooth movement between individuals for the same applied force has been noted in studies involving humans (3, 8, 14–17) and animals (18, 19) even between littermates. These findings suggest strongly that individual-specific characteristics are important to the biologic responses that result in bone remodeling when orthodontic forces are applied. A number of *in vivo* studies have measured cytokine production in gingival crevicular fluid (GCF) during human tooth movement in attempt to uncover and quantify some of these biologic responses (for reviews: 20, 21). These studies were limited, however, by the use of uncontrolled force systems, a failure to quantify movement in three-dimensions, and short durations of investigation.

Controlled tooth movement in humans using similar protocols in three previous studies (3, 8, 14) showed clinically important and statistically significant results from each study. Combined data offer information from 50 maxillary canines moved using 4–52 kPa for 84 days and suggest 26 kPa as optimal and 0.063 mm/day as the associated maximum mean speed of tooth movement. However, these data also demonstrate mean speeds for the same stress were about two times higher in subjects who showed growth compared with subjects who showed no growth and over five times faster in some subjects compared with others.

The rate of tooth movement depends on the rate of bone resorption, which involves a complex cascade of events and agents that act synergistically and antagonistically in a highly redundant system. Interleukin-1 (IL-1), a pro-inflammatory cytokine, and its competitive antagonist, interleukin-receptor antagonist (IL-1RA), are just one pair of these agents (see reviews in 8, 14). Nevertheless, the ratio of IL-1β/IL-1RA measured in GCF during tooth movement at control vs. experimental sites (activity index; AI) accounted for 60% of the variation shown for speed of movement among the 50 teeth studied. Relative amounts of these cytokines have been linked to certain IL-1 gene cluster polymorphisms, thus, the genotype of recent subjects were investigated. Initial results demonstrated that subjects with specific IL-1 gene cluster polymorphisms showed significantly faster tooth movement (14).

Data from a fourth, previously unpublished study (22), using a similar protocol were combined with those from the three previous studies and are reported herein. These data were used to investigate factors that may account for differences in rates of human bone remodeling and tooth movement.

Materials and methods

Thirty-four generally healthy subjects gave informed consent to participate in accordance with ethical standards of the appropriate institutional review board. All subjects had orthodontic treatment plans involving extraction of maxillary first premolars and distal movement of maxillary canines. Subjects were instructed to maintain good oral hygiene and avoid medications during the study. One subject (4M1) violated the latter criterion and was withdrawn. Data were, therefore, based on 21 females and 12 males with starting mean age 14.8 ± 3.9 years (Table 1). Detailed protocols were reported previously (3, 8, 14) and will be represented in brief.

Segmental mechanics were used to translate distally 66 maxillary canines from day 0 to 84, while the mandibular teeth were without appliances. For passive anchorage maxillary first molars were linked with a

Table 1. **Demographics of subjects; side, stress, speed of distal movement of maxillary canine; R^2 of distal movement vs. time; and average activity index (AI)**

Subject	Age (years)	Growth status	Side	Stress (kPa)	Speed (mm/day)	R^2	Average AI
4F1	13.3	Grower	R	52	0.079	0.973	0.70
			L	78	0.090	0.988	0.53
4F2	12.8	Grower	R	78	0.059	0.949	0.55
			L	52	0.071	0.940	0.89
4F3	12.2	Grower	R	26	0.037	0.941	Not determined
			L	78	0.072	0.979	
4F4	11.8	Grower	R	78	0.109	0.979	Not determined
			L	13	0.075	0.978	
4F5	17.9	Non-Grower	R	26	0.028	0.979	Not determined
			L	78	0.029	0.872	
4F6	10.8	Grower	R	78	0.061	0.962	Not determined
			L	52	0.048	0.986	
4M2	14.2	Grower	R	78	0.065	0.980	Not determined
			L	13	0.061	0.983	
4M3	16.1	Grower	R	13	0.037	0.952	Not determined
			L	78	0.051	0.920	
3F1	16.1	Grower	R	26	0.067	0.980	1.08
			L	52	0.060	0.988	0.55
3F2	13.2	Grower	R	26	0.060	0.983	0.78
			L	52	0.065	0.988	1.48
3F3	24.6	Non-Grower	R	26	0.062	0.986	0.36
			L	52	0.066	0.991	0.36
3F4	11.5	Grower	L	26	0.091	0.992	1.52
			R	52	0.081	0.988	0.95
3F5	15.2	Grower	L	13	0.049	0.994	0.48
			R	26	0.070	0.994	0.69
3M1	12.5	Grower	R	26	0.072	0.983	1.20
			L	52	0.059	0.989	0.94
3M2	13.8	Grower	L	26	0.097	0.988	2.43
			R	52	0.084	0.990	0.95
3M3	12.2	Grower	R	26	0.090	0.984	2.02
			L	52	0.080	0.989	2.34
3M4	16.3	Grower	R	26	0.054	0.957	0.69
			L	52	0.034	0.991	0.41
3M5	14.1	Grower	L	13	0.046	0.990	0.43
			R	26	0.058	0.993	0.39
2F1	30.9	Non-Grower	R	13	0.012	0.659	0.21
			L	26	0.013	0.729	0.46
2F2	15.1	Non-Grower	L	13	0.021	0.881	1.54
			R	26	0.022	0.926	0.90
2F3	16.1	Non-Grower	R	13	0.033	0.957	0.75
			L	52	0.052	0.884	1.08

Table 1. **Continued**

Subject	Age (years)	Growth status	Side	Stress (kPa)	Speed (mm/day)	R^2	Average AI
2F4	12.8	Grower	L	13	0.052	0.901	1.33
			R	26	0.053	0.841	1.23
2F5	10.4	Grower	R	13	0.045	0.864	0.72
			L	52	0.056	0.948	0.83
2M1	17.9	Non-Grower	L	13	0.015	0.881	0.77
			R	52	0.037	0.760	0.98
2M2	12.9	Grower	R	13	0.057	0.905	2.59
			L	26	0.043	0.933	0.97
2M3	14.2	Grower	L	13	0.068	0.963	0.79
			R	52	0.063	0.924	1.01
1F1	12.2	Grower	R	4	0.029	0.968	0.50
			L	13	0.046	0.970	1.62
1F2	14.8	Grower	R	4	0.020	0.970	1.01
			L	13	0.018	0.913	0.99
1F3	13.2	Grower	R	4	0.048	0.985	1.09
			L	13	0.049	0.941	0.86
1F4	13.3	Grower	L	4	0.019	0.857	0.84
			R	13	0.052	0.970	0.74
1F5	14.4	Grower	L	4	0.022	0.903	3.62
			R	13	0.026	0.990	7.59
1M1	16.2	Grower	L	4	0.016	0.725	0.93
			R	13	0.024	0.903	1.12
1M2	13.9	Grower	L	4	0.042	0.868	0.26
			R	13	0.066	0.929	0.62

The subjects are identified by study #, sex (F = female, M = male), subject #.

Nance appliance and posterior teeth were linked in each maxillary quadrant via buccal stainless-steel segment archwires of rectangular cross-section (≥0.016 × 0.018 inch) plus figure-of-eight ligation (Fig. 1A, B). Prior to day 0 by approximately: 1 month, each subject received anchorage appliances and began twice-daily chorhexidine gluconate oral rinses; 2 weeks, maxillary first premolars were removed. Forces and counter-moments were delivered to each maxillary canine starting on day 0 using a stainless-steel vertical loop auxiliary wire of rectangular cross-section (≥0.016 × 0.018 inch) ligated to the canine bracket and extending through the auxiliary tube on the first molar band in the same quadrant (Fig. 1A, B). Each loop was activated by a nickel–titanium spring calibrated at mouth-temperature and selected to apply 4, 13, 26, 52, or 78 kPa to a given canine. Corresponding forces were approximately 18, 60, 120, 240, and 360 cN, respectively. Two different stresses per subject were assigned systematically via a balanced incomplete block design, with stresses assigned randomly to right or left sides.

Subjects made nine to 10 visits, on days 0, 1, 3, ±7, 14, 28, 42, 56, 70, and 84. At each visit subjects had oral hygiene and gingival inflammation evaluated using the modified gingival index (MGI) (23), GCF samples collected, a supragingival prophylaxis performed, and a maxillary dental impression made in polyvinylsiloxane using a custom tray.

Established techniques were used for collection, storage, and analysis of GCF (8, 14). At each visit, two GCF samples were obtained from two experimental sites, distal of each maxillary canine, and one control site, interproximal of a mandibular canine, or adjacent tooth. The two samples per site were combined and assayed using commercial kits and a spectrophotometric micro-plate reader to quantify IL-1β (Cayman Chemical, Ann Arbor, MI, USA) and IL-1RA (R & D

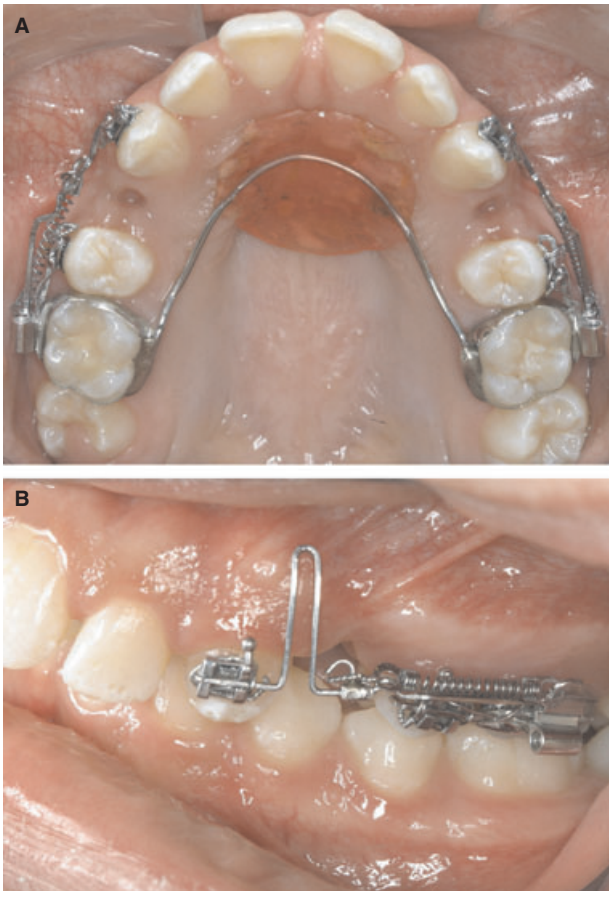

Fig. 1. (A) Subject 4M2: occlusal view showing appliances including vertical loops activated by calibrated springs selected to deliver a prescribed force (F) for a specified stress (σ) to each maxillary canine, according to: $\sigma = F/A_a$, where $A_a = L_r a(1 - [b^2/a^2])^{½}$ was the distal root surface area of the canine adjusted for root curvature, and labiolingual ($2a$) and mesiodistal ($2b$) widths at the cemento-enamel junction plus the root length (L_r) were measured from a periapical radiograph of the tooth corrected for magnification (3). (B) Subject 4M2: left buccal view showing heights matched for the center of the vertical loop and center of resistance of the maxillary canine (C_R), estimated using: $C_R = 0.24L_r$.

Systems, Minneapolis, MN, USA). GCF samples from 25 of 33 subjects were similarly assayed to quantify total protein (BCA Protein Assay; Pierce Biotechnology, Rockford, IL, USA). Results of duplicate enzyme-linked immunosorbent assays (ELISA) for each cytokine and total protein were averaged for each time-point. Readings below the detectable limit were not used. IL-1β and IL-1RA levels at each visit were expressed as ratios of experimental vs. control sites (E/C), relative to total protein (where able), and via a modified AI as previously described (8, 14), where:

$$\text{AI} = \text{Experimental}\frac{\dfrac{\text{IL-1}\beta}{\text{IL-1RA}}}{\text{Control}\dfrac{\text{IL-1}\beta}{\text{IL-1RA}}}$$

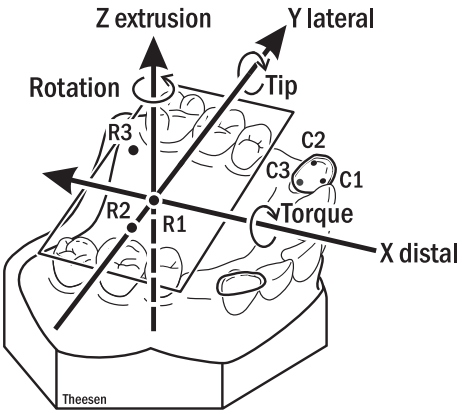

Fig. 2. Diagram of a maxillary dental model and 3 custom acrylic templates: one for the posterior anchor teeth and one for each maxillary canine. Three markers were embedded in each template: R1, R2, R3 in the posterior template defined the origin and 3 orthogonal axes (X, Y, Z); C1, C2, C3 in canine templates allowed serial measurements of canines in terms of linear positions (distal, lateral, extrusion) relative to the origin and angular positions (tip, torque, rotation) relative to the axis system (3).

Overall average values for IL-1β and IL-1RA levels and AI for each maxillary canine were calculated from day 0 to 84 or until retraction was complete according to the following: initial averages and standard deviation (SD) for all GCF measures were calculated over all time-points for each tooth; any data >2 SD above or below the initial average for a given site or tooth were defined as outlier data; averages and SD for measures, sites, and teeth were recalculated excluding outlier data; and average values were determined based on remaining data from four to nine time-points, where average number of time-points was 7 ± 1.

Tooth movements were quantified using a microscope (MM-11 Measurescope; Nikon Inc., Melville, NY, USA), the series of nine to 10 maxillary dental casts per subject derived from impressions made at each visit, and a set of three custom acrylic templates for each subject (Fig. 2). Repeated measurement errors for this technique were a maximum of 0.05 mm and 0.28°.

Cheek-wipe samples were collected from 18 of 33 subjects for genotyping (Kimball Genetics, Denver, CO or Medical University of South Carolina) of IL-1 gene cluster polymorphisms at loci: IL-1A(+4845), IL-1B(+3954), and IL-1RN (variable number of tandem repeats of 86 base pairs; $VNTR_{86}$), using previously described techniques (14, 22).

Growth status for each subject was determined as positive (grower) or negative (non-grower) by presence

or absence of demonstrated height change and craniofacial growth via serial lateral cephalometric superimpositions during orthodontic treatment.

For each maxillary canine, three linear (distal, lateral, and extrusion), and three angular (distal crown tip, lateral crown torque, and distopalatal rotation) movements (Fig. 2) were plotted vs. time and compared to test if distal tooth translation predominated. Average values of movements for each stress were plotted vs. approximate time-points because 10 subjects had at least one visit that was 1–7 days different from the planned sequence. Speed was calculated from the slope of distal movement vs. time for each maxillary canine. Linear regression and correlation analyses determined strength of the relationships between speed of tooth movement, cytokine levels (IL-1β, IL-1RA, AI), genotype, sex, growth status, and stress. Repeated measures ANCOVA with tests for class and regression effects were conducted with statistical significance set at $p < 0.05$.

Results

Subjects had average MGI scores of ≤0.3, indicating good oral hygiene with no or minimal visible signs of inflammation. Posterior tooth positions were preserved (≤1.0 mm change) for all subjects as verified by cephalometric superimpositions and the generally good fit of the posterior template on all casts for a subject. Twenty-seven subjects were growers (16 females, 11 males), and six subjects (five females, one male) were non-growers (Table 1). The number of teeth moved per stress and growth status of the subjects (growers, non-growers) were: 4 kPa: 7, 0; 13 kPa: 15, 5; 26 kPa: 12, 4; 52 kPa: 12, 3; 78 kPa: 6, 2.

Retraction ended for five teeth in four subjects by: day 70 for 1F4, right; 4F1, left; 4F3, left; 4F4, left; and day 56 for 4F4, right. Average maximum distal movements (±SD) during the study were: 2.41 (±1.22), 3.63 (±1.53), 5.04 (±2.15), 5.31 (±1.24), and 5.10 (±1.07) mm, for 4, 13, 26, 52, and 78 kPa, respectively. At day 1, average (±SD) distal movement ranged between 0.14 (±0.10) and 0.43 (±0.29) mm for the four stresses, followed by relatively steady (linear) distal movement over time at all stresses (Fig. 3) with average $R^2 = 0.938$ (Table 1). Plots of distal movement vs. time for individual teeth showed the same general pattern in 63 cases. Three teeth (1M2, left; 2M1, right; 4M3, left)

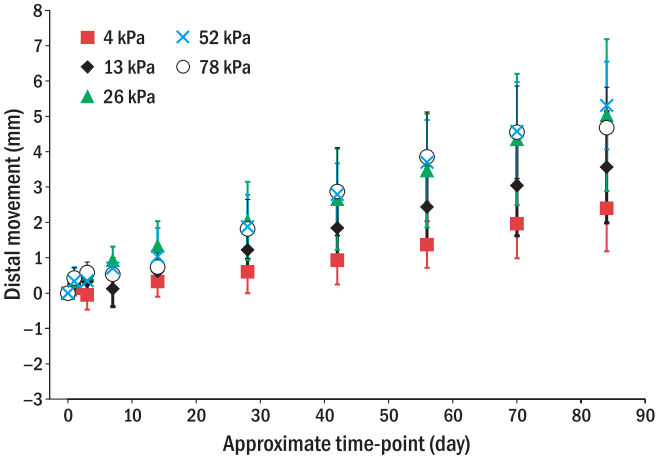

Fig. 3. Average distal movement of maxillary canines vs. approximate time-point for 4 applied stresses. Vertical lines indicate 1 SD about average.

demonstrated a lag phase between days 3 and 28, whereby distal movement at ≥1 time-point in this period was equal to or less than that at day 1, and after which the speed of tooth movement was linear and markedly increased from days 42 to 84 (data not shown).

Speeds of distal tooth movement ranged between 0.016 and 0.109 mm/day in growers and 0.012–0.066 mm/day in non-growers. Maximum difference in speed between all teeth in the study was 9.1:1. For the same stress and growth status, maximum differences in speed were 4.2:1 for 13 kPa in growers and 4.8:1 for 26 kPa in non-growers. Average speeds of distal movement (±SD) were 0.028 (±0.012), 0.043 (±0.019), 0.057 (±0.024), 0.062 (±0.015), and 0.067 (±0.024) mm/day for 4, 13, 26, 52, and 78 kPa, respectively (Fig. 4). On average, speed increased approximately

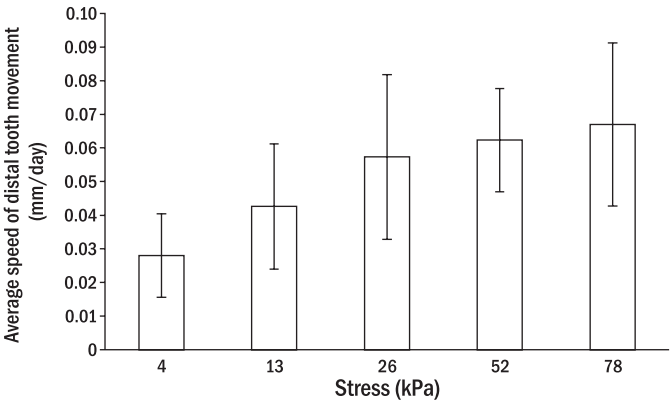

Fig. 4. Average speed of distal movement of maxillary canines vs. applied stress. Vertical lines indicate 1 SD about average.

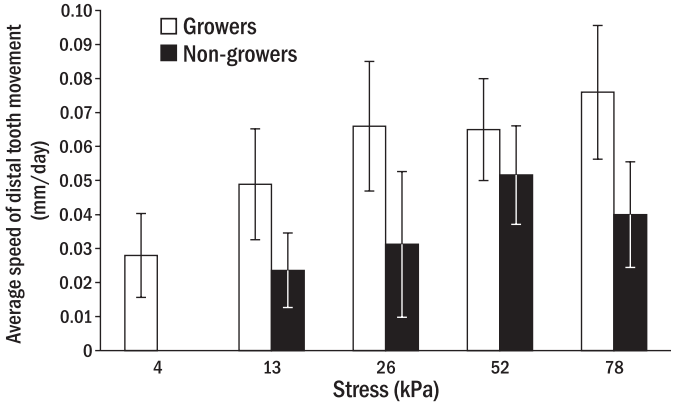

Fig. 5. Average speed of distal movement of maxillary canines vs. applied stress and growth status of subjects. Vertical lines indicate 1 SD about average.

linearly with stress over this range. However, the effect of stress was not statistically significant ($p > 0.05$). Average speeds of distal movement in growers were faster than in non-growers at each stress (Fig. 5) but this effect was also not statistically significant ($p > 0.05$).

Extrusion and angular movements tended to fluctuate with time (Fig. 6A–D). These movements were generally small for applied stresses from 4 to 52 kPa; where absolute values of averages were ≤0.71 mm for extrusion and ≤5.34° for all three angular movements. Movements were generally larger for 78 kPa; where absolute averages were: ≤1.05 mm for extrusion (Fig. 6A), ≤5.38° for distal crown tip (Fig. 6B), ≤6.38° for lateral crown torque (Fig. 6C), and ≤13.75° for distopalatal rotation (Fig. 6D). Lateral movements tended to steadily increase with time (Fig. 6E), similar to but lesser than distal movements, and were highest for 78 kPa where absolute averages were ≤2.79 mm, whereas these were ≤1.81 mm for 4–52 kPa.

In general, as previously reported (8, 14), the levels of IL-1β and IL-RA in GCF fluctuated over time for all subjects and amounts of cytokines collected in GCF samples were generally low (Table 2). Malfunction of an assay for IL-1β for subject 4F6 and the screening protocol for outlier data resulted in average AI calculations for 27 of 33 subjects (Table 1).

Genotypes for IL-1A(+4858), IL-1B(+3954), and IL-1RN($VNTR_{86}$) loci for 18 subjects (Table 2) were grouped according to: genotype 1, homozygous for allele 1 (1, 1); genotype 2, heterozygous (1,2) or homozygous for allele 2 (2,2); and genotype 3, having alleles 1 and 3. For the IL-1A(+4858) locus, eight subjects were genotype 1 while 10 subjects were genotype 2. For the IL-1B(+3954) locus, nine subjects each were genotypes 1 and 2. For the IL-1RN locus, 10 subjects were genotype 1, seven subjects were genotype 2, and one subject was genotype 3.

A step-wise regression analysis using speed of distal tooth movement as the dependent variable showed three significant factors affected speed at the 15% level: IL-1B(+3954) genotype ($p = 0.039$), AI ($p = 0.0005$), and IL-1RA in GCF at the experimental site ($p = 0.005$). That is, higher speeds were significantly associated with genotype 2 at IL-1B(+3954), higher AI, and lower IL-1RA in GCF at the experimental site. Combined, these three factors provided a model that explained 69% of the variability found in the speed of distal tooth movement (Fig. 7).

Discussion

Sixty-six maxillary canines in 33 human subjects were retracted into edentulous spaces by continuous stresses of 4, 13, 26, 52, or 78 kPa for 84 days. Tooth movement was predominantly distal (Fig. 3), where the relatively large amount shown at day 1 likely represented initial squeezing of the periodontal ligament in response to the applied force. Generally steady distal movement was demonstrated by 95% of the teeth from days 3 to 84 or until retraction was complete. Only three teeth, one each moved by 4, 52, and 78 kPa, showed a so-called 'lag phase' from days 3 to 28, after which time these teeth also showed a linear relationship between distal movement and time. Contrary to previous suggestion based on preliminary data (8), current evidence does not support the theory that presence of a lag phase is related to higher stresses. Average lateral tooth movement also showed a steady but smaller increase with respect to time (Fig. 6E) and could be accounted for by cases in which initial dental arch form and position of the maxillary canine necessitated some lateral as well as distal movement to approximate contact points on the distal of the canine and mesial of the second premolar in the same quadrant (e.g. Fig. 1A). Tracking tooth movement relative to an orthogonal axis system, as in the current protocol, tends to underestimate the total amount of movement during canine retraction. The fluctuations with respect to time and relatively small amounts of extrusion and

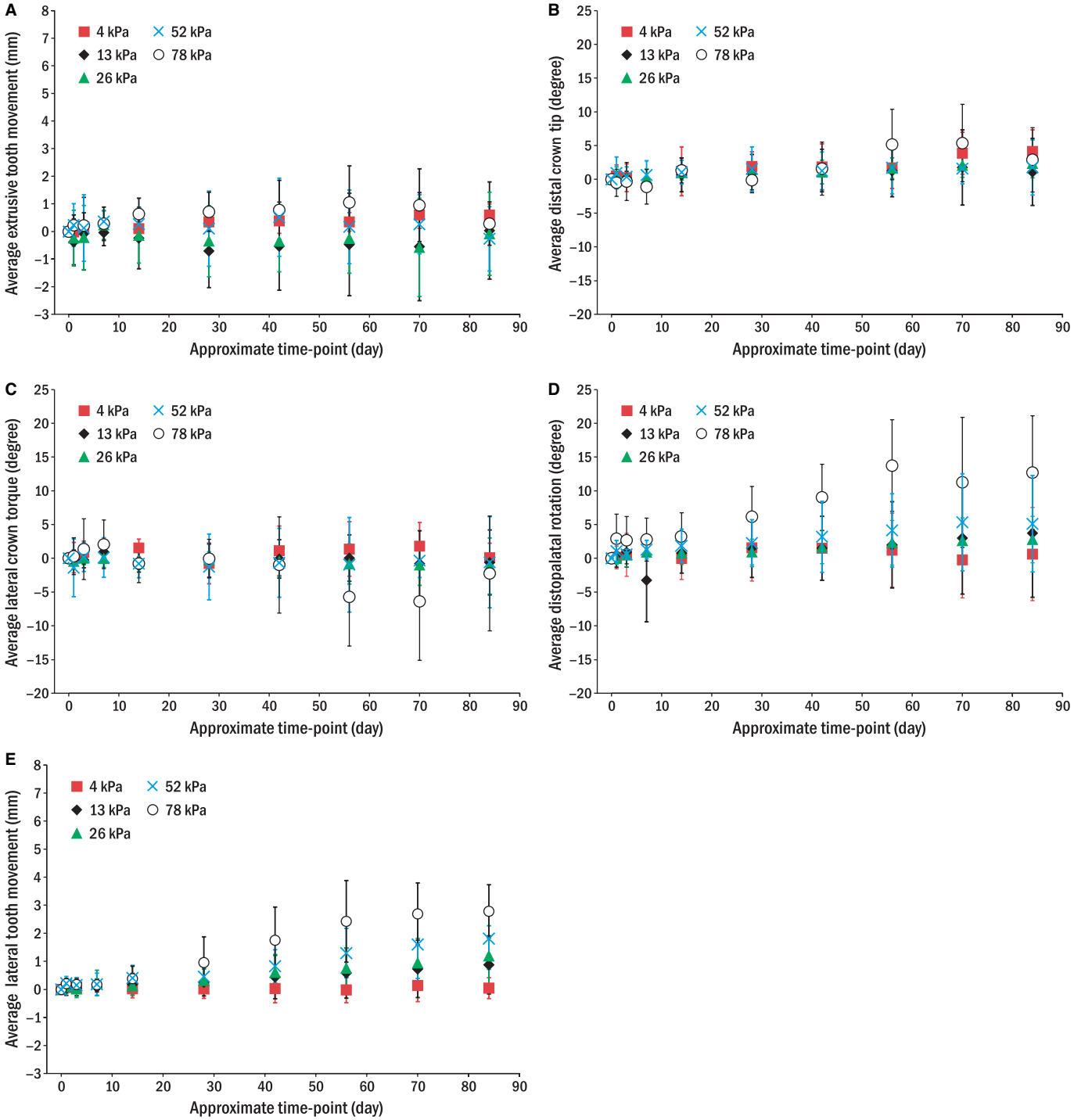

Fig. 6. Average movement of maxillary canines vs. approximate time-point for 4 applied stresses: (A) extrusive, (B) distal crown tip, (C) lateral crown torque, (D) distopalatal rotation, (E) lateral. Vertical lines indicate 1 SD about average.

angular movements suggest that predictable tooth translation was possible in general, via the applied mechanics used.

Average tooth movements in all aspects were larger for 78 kPa than lower stresses. Furthermore, average speed of distal movement showed a positive linear relationship with stress for 4–78 kPa. However, the effect of stress on speed of distal movement was not significant for these combined data. Overall, teeth in growers moved faster than teeth in non-growers. However, the effect of growth status on speed was also not significant for these combined data. Three factors

Table 2. **Subjects; side, stress, average IL-1β, and IL-1RA in GCF at experimental site (E) relative to protein and relative to control site (C); and genotype (allele numbers)**

			IL-1β		IL-1RA		Genotype		
Subject	Side	Stress (kPa)	pg/μg protein	E/C	pg/μg protein	E/C	IL-1A (+4858)	IL-1B (+3954)	IL-1RN ($VNTR_{86}$)
4F1	R	52	1.11	1.30	16.16	1.80	1,2	1,2	1,1
	L	78	0.85	0.67	17.24	1.63			
4F2	R	78	0.48	0.54	21.22	0.96	1,2	1,1	1,3
	L	52	0.53	0.89	17.72	0.84			
4F3	R	26	0.31	2.06	6.40	1.63	1,1	1,2	1,1
	L	78	0.44	3.95	7.57	2.38			
4F4	R	78	0.68	2.34	31.50	0.99	1,2	1,2	1,1
	L	13	0.73	2.75	27.38	1.38			
4F5	R	26	ND	ND	ND	ND	1,2	1,1	2,2
	L	78	ND	ND	ND	ND			
4F6	R	78	ND	ND	12.83	0.86	1,1	1,1	1,1
	L	52	ND	ND	19.68	0.83			
4M2	R	78	1.66	1.29	39.49	0.55	1,2	1,2	1,1
	L	13	2.58	2.94	66.14	0.96			
4M3	R	13	0.20	ND	23.86	1.18	1,1	1,1	1,1
	L	78	0.52	4.91	21.39	1.30			
3F1	R	26	0.65	0.34	19.89	0.38	1,2	1,1	2,2
	L	52	0.37	0.28	20.70	0.46			
3F2	R	26	0.26	0.74	34.13	0.52	1,1	1,1	1,2
	L	52	0.22	0.93	30.44	0.60			
3F3	R	26	0.95	0.24	23.47	0.76	1,1	1,1	1,2
	L	52	0.74	0.24	22.22	0.64			
3F4	L	26	1.71	0.77	15.90	0.64	1,1	1,2	1,1
	R	52	2.09	0.64	19.05	0.81			
3F5	L	13	2.01	0.94	72.25	2.91	2,2	2,2	1,2
	R	26	4.27	2.14	56.43	2.30			
3M1	R	26	2.49	0.98	24.29	1.10	1,1	1,1	1,2
	L	52	1.69	0.63	24.43	1.24			
3M2	L	26	2.52	2.21	16.32	0.84	1,2	1,2	1,1
	R	52	1.18	1.01	22.32	1.27			
3M3	R	26	0.31	1.91	16.01	0.98	1,2	1,2	1,1
	L	52	0.63	3.85	27.72	2.15			
3M4	R	26	0.77	0.34	25.82	0.62	1,1	1,1	2,2
	L	52	0.45	0.21	22.07	0.51			
3M5	L	13	2.82	0.60	35.94	1.69	1,2	1,2	1,1
	R	26	2.72	0.63	33.70	2.49			
2F1	R	13	ND	0.51	ND	1.22	ND	ND	ND
	L	26	ND	1.32	ND	1.48			
2F2	L	13	ND	1.37	ND	1.54	ND	ND	ND
	R	26	ND	1.25	ND	1.67			

Table 2. **Continued**

			IL-1β		IL-1RA		Genotype		
Subject	Side	Stress (kPa)	pg/μg protein	E/C	pg/μg protein	E/C	IL-1A (+4858)	IL-1B (+3954)	IL-1RN ($VNTR_{86}$)
2F3	R	13	ND	0.90	ND	0.74	ND	ND	ND
	L	52	ND	1.16	ND	0.83			
2F4	L	13	ND	1.19	ND	0.94	ND	ND	ND
	R	26	ND	1.16	ND	1.27			
2F5	R	13	ND	0.87	ND	0.85	ND	ND	ND
	L	52	ND	0.99	ND	0.98			
2M1	L	13	ND	1.21	ND	1.03	ND	ND	ND
	R	52	ND	1.26	ND	1.01			
2M2	R	13	ND	5.20	ND	0.98	ND	ND	ND
	L	26	ND	1.12	ND	1.17			
2M3	L	13	ND	1.89	ND	1.41	ND	ND	ND
	R	52	ND	1.46	ND	1.04			
1F1	R	4	0.48	0.68	50.95	1.01	ND	ND	ND
	L	13	1.83	1.77	47.09	0.97			
1F2	R	4	1.09	1.41	91.98	1.32	ND	ND	ND
	L	13	1.04	1.17	102.86	1.42			
1F3	R	4	2.10	0.95	61.39	0.93	ND	ND	ND
	L	13	2.58	1.25	74.82	1.18			
1F4	L	4	1.32	1.08	83.53	1.30	ND	ND	ND
	R	13	1.07	0.90	69.03	1.08			
1F5	L	4	3.46	4.37	67.13	1.03	ND	ND	ND
	R	13	4.16	7.60	91.00	1.45			
1M1	L	4	1.04	1.79	144.82	3.19	ND	ND	ND
	R	13	0.90	1.66	100.35	2.23			
1M2	L	4	0.29	0.23	69.31	2.14	ND	ND	ND
	R	13	1.22	1.63	101.49	2.58			

The subjects are identified by study #, sex (F = female, M = male), subject.
E, experimental site; C, control site; $VNTR_{86}$, variable number of tandem repeats of 86 base pairs; ND, not determined.

that were shown to have a significant effect on speed of distal movement were: IL-1B(+3954) genotype, average AI, and IL-1RA in GCF at the experimental site during the tooth movement. These results are in general agreement with previous findings on smaller subsamples (8, 14, 22). The results are also consistent with reports that individuals with genotype 2 compared with genotype 1 at IL-1B(+3954) secrete more IL-1β for the same stimulus (24). Such individuals might be expected to have higher average AI values and relatively lower average IL-1RA levels in GCF at experimental sites as found in the current study, and associated with increased relative bone resorption and faster speeds of tooth movement.

Limitations of the study protocol were discussed previously (3, 8, 14) and include the indirect assessment of bone turnover agents by measuring GCF, limited focus on only two of such agents and genes responsible for their production, potential for unmeasured effects on appliances such as binding of active components, and challenges in obtaining consistent results from ELISA. It should be further noted that evidence from studies of single nucleotide polymorphisms (SNPs), such as those of the IL-1 gene cluster,

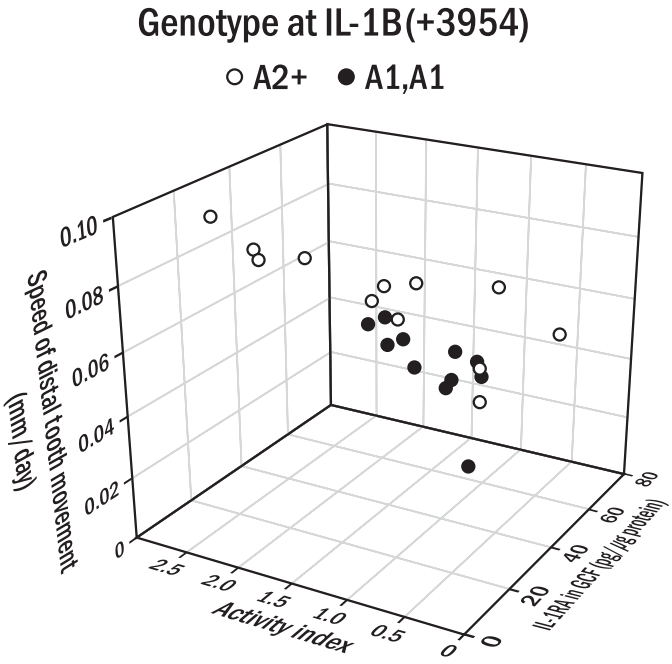

Fig. 7. Quasi-three-dimensional graph showing effects of IL-1B genotype [○ subjects homozygous for allele 1 (A1,A1); • subjects with ≥1 copy of allele 2 (A2+)], activity index (AI), and IL-1RA in GCF at experimental sites on speed of distal movement of maxillary canines.

demonstrate regional and ethnic differences in allelic frequencies (e.g. 25) and the interplay between SNPs (e.g. 26). Genes do not act in isolation; there is growing body of evidence that epistasis – modification of the action of a gene by one or more other genes – plays a significant role (27). This suggests future investigations include larger samples and gene interactions. Of additional note, genetics associated with normal physiologic processes such as bone remodeling, in generally healthy individuals, like most orthodontic patients, has received limited study. Furthermore, unlike genetically complex diseases, many of the candidate environmental factors associated with the phenotype: speed of bone turnover and orthodontic tooth movement, can be identified and measured (28). Thus, orthodontic tooth movement could provide a well-controlled human model for the study of the molecular biology and genetics of bone.

Conclusions

Combined data from 66 teeth moved over 84 days demonstrated further that translation is possible and that a number of factors affect the speed of bone remodeling and tooth movement in humans. Specifically, having at least one copy of allele 2 at IL-1B(+3954), high average AI, and low average IL-1RA in GCF at experimental sites are associated with faster distal tooth movement. Higher stresses in the range 4–78 kPa and evidence of growth also may be related to faster tooth movement.

Clinical relevance

Factors that maximize speed of tooth movement and explain inter-individual variability remain unknown. Research reported herein examines mechanical, biologic, and genetic factors that may account for differences in speed of bone remodeling and tooth movement between patients. The results provide a basis for improved future clinical efficiency through measurement of applied forces and diagnostic and therapeutic predictors of patient-responses.

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References

1. Quinn RS, Yoshikawa DK. A reassessment of force magnitude in orthodontics. *Am J Orthod* 1985;88:252–60.
2. Ren Y, Maltha JC, Kuijpers-Jagtman AM. Optimum force magnitude for orthodontic tooth movement: a systematic literature review. *Angle Orthod* 2003;73:86–92.
3. Iwasaki LR, Haack JE, Nickel JC, Morton J. Human tooth movement in response to continuous stress of low magnitude. *Am J Orthod Dentofacial Orthop* 2000;117:175–83.
4. Lee BW. Relationship between tooth-movement rate and estimated pressure applied. *J Dent Res* 1965;44:1053.
5. Lee BW. The force requirements for tooth movement. Part II: Uprighting and root torque. *Aust Orthod J* 1995;14:34–9.
6. Storey E, Smith R. Force in orthodontics and its relation to tooth movement. *Aust J Dent* 1952;56:11–8.
7. Darendeliler MA, Darendeliler H, Uner O. The drum spring (DS) retractor: constant and continuous force for canine retraction. *Eur J Orthod* 1997;19:115–30.
8. Iwasaki LR, Crouch LD, Tutor A, Gibson S, Hukmani N, Marx DB et al. Tooth movement and cytokines in gingival crevicular fluid and whole blood in growing and adult subjects. *Am J Orthod Dentofacial Orthop* 2005;128:483–91.
9. Bridges T, King G, Mohammed A. The effect of age on tooth movement and mineral density in the alveolar tissues of the rat. *Am J Orthod Dentofacial Orthop* 1988;93:245–50.

10. Kyomen S, Tanne K. Influences of aging changes in proliferative rate of PDL cells during experimental tooth movement in rats. *Angle Orthod* 1997;67:67–72.
11. Misawa-Kageyama Y, Kageyama T, Moriyama K, Kurihara S, Yagasaki H, Deguchi T et al. Histomorphometric study on the effects of age on orthodontic tooth movement and alveolar bone turnover in rats. *Eur J Oral Sci* 2007;115:124–30.
12. Ren Y, Maltha JC, Van 't Hof MA, Kuijpers-Jagtman AM. Age effect on orthodontic tooth movement in rats. *J Dent Res* 2003;82:38–42.
13. Takano-Yamamoto T, Kawakami M, Yamashiro T. Effect of age on the rate of tooth movement in combination with local use of 1,25(OH)2D3 and mechanical force in the rat. *J Dent Res* 1992;71:1487–92.
14. Iwasaki LR, Gibson CS, Crouch LD, Marx DB, Pandey JP, Nickel JC. Speed of tooth movement is related to stress and IL-1 gene polymorphisms. *Am J Orthod Dentofacial Orthop* 2006;130:698e1–9.
15. Owman-Moll P, Kurol J, Lundgren D. Continuous versus interrupted continuous orthodontic force related to early tooth movement and root resorption. *Angle Orthod* 1995;65:395–401; discussion 01–2.
16. Owman-Moll P, Kurol J, Lundgren D. The effects of a four-fold increased orthodontic force magnitude on tooth movement and root resorptions. An intra-individual study in adolescents. *Eur J Orthod* 1996;18:287–94.
17. Owman-Moll P, Kurol J, Lundgren D. Effects of a doubled orthodontic force magnitude on tooth movement and root resorptions. An inter-individual study in adolescents. *Eur J Orthod* 1996;18:141–50.
18. Mitchell DL, Boone RM, Ferguson JH. Correlation of tooth movement with variable forces in the cat. *Angle Orthod* 1973;43:154–61.
19. Pilon JJ, Kuijpers-Jagtman AM, Maltha JC. Magnitude of orthodontic forces and rate of bodily tooth movement. An experimental study. *Am J Orthod Dentofacial Orthop* 1996;110:16–23.
20. Iwasaki LR, Nickel JC. Markers of paradental tissue remodeling in the gingival crevicular fluid of orthodontic patients. In: Krishnan V, Davidovitch Z, editors. *Biological Mechanisms of Tooth Movement*. Oxford: Wiley-Blackwell; 2009, pp. 123–42.
21. Ren Y, Vissink A. Cytokines in crevicular fluid and orthodontic tooth movement. *Eur J Oral Sci* 2008;116:89–97.
22. Chandler JR. *Stress Magnitude and Interleukin-1 Affect the Speed of Orthodontic Tooth Movement in Humans*. Master's thesis, Lincoln, NE: University of Nebraska Medical Center; 2006.
23. Lobene RR, Weatherford T, Ross NM, Lamm RA, Menaker L. A modified gingival index for use in clinical trials. *Clin Prev Dent* 1986;8:3–6.
24. Cork MJ, Tarlow JK, Clay FE, Crane A, Blakemore AI, McDonagh AJ et al. An allele of the interleukin-1 receptor antagonist as a genetic severity factor in alopecia areata. *J Invest Dermatol* 1995;104:15S–6S.
25. Armitage GC, Wu Y, Wang H-Y, Sorrell J, di Giovine FS, Duff GW. Low prevalence of a periodontitis-associated interleukin-1 composite genotype in individuals of Chinese heritage. *J Periodontol* 2000;71:164–71.
26. Chen H, Wilkins LM, Aziz N, Cannings C, Wyllie DH, Bingle C et al. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. *Hum Mole Genet* 2006;15:519–29.
27. Phillips PC. Epistasis – the essential role of gene interactions in the structure and evolution of genetic systems. *Nat Rev Genet* 2008;9:855–67.
28. Iwasaki LR, Crouch LD, Nickel JC. Genetic factors and tooth movement. *Sem Orthod* 2008;14:135–45.