of Wnt signaling, β-catenin levels are kept low through binding with the axin–adenomatous polyposis coli complex, phosphorylation by casein kinase 1 and glycogen synthase kinase 3 and ubiquitination by Skp-Cul1-F-box-beta-transducin repeats-containing proteins. The binding of Wnt to its receptors prevents glycogen synthase kinase 3 phosphorylation of β-catenin, releasing it from the axin–adenomatous polyposis coli complex. The stabilized β-catenin accumulates in the cytosol, enters the nucleus to interact with the lymphoid enhancer factor/T cell factor transcription factor and activates transcription of Wnt signaling (31). The activated Wnt signaling enhances expression of *Bmp-2, -4 and -6*, leading to the stimulation of cytodifferentiation and mineralization of MPDL22 cells.

A number of studies have demonstrated that post-transcriptional modifications of protein, such as phosphorylation, acetylation and ubiquitination, regulate osteoblastic differentiation. *Runx2* is a master gene in osteoblastic differentiation (32,33) and is regulated by several post-transcriptional modifications. BMP-2 regulates *Runx2* transactivation by the phosphorylation of Smad1/5/8 and p38 to induce osteoblastic differentiation (34). SMURF1 induced the ubiquitin-mediated degradation of Smad1/5 and RUNX2 to inhibit the formation of osteoblastic bone (35,36). BMP-2 stimulates acetylation of the transcriptional cofactor, p300-dependent RUNX2, leading to the activation of transcription and the inhibition of SMURF1-mediated proteasomal degradation of RUNX2 (37). Hence, post-transcriptional modifications of protein are essential for osteoblastic differentiation.

This work is the first to report that bortezomib enhances the cytodifferentiation and mineralization of PDL cells by increasing the accumulation of β-catenin and the expression of *Bmp-2*, *-4* and *-6* mRNAs. In addition, we found that bortezomib enhances BMP-2-induced cytodifferentiation and mineralization of PDL tissues. *In-vivo* experiments are now essential to investigate the use of bortezomib in periodontal regeneration therapy. Considering that the PDL tissue plays a pivotal role in the homeostasis, repair and regeneration of periodontal tissues, these data indicate that bortezomib is an important compound for periodontal regenerative therapy.

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Influence of genetic and environmental factors on oral diseases and function in aged twins

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SUMMARY **This study was conducted to quantify the genetic and environmental contributions to oral disease and function in twins. Participants were middle-aged and old twins, 116 monozygotic and 16 dizygotic pairs whose mean age was 66·1 ± 10·3 (SD) years. Number of teeth, percentage of decayed, filled and missing teeth and periodontal status were recorded as indicators of oral disease. The widths of upper and lower dental arch served as indicators of morphological figures. Furthermore, stimulated salivary flow rate, occlusal force and masticatory performance were measured as indicators of oral function. Univariate genetic analysis with monozygotic and dizygotic twin pairs was conducted to detect the fittest structural equation model of each outcome. Both number of teeth and periodontal status fitted the model composed of common environmental factor and unique environmental factor. Decayed, filled and missing teeth, morphological figures and measurements of oral function fitted the model composed of additive genetic factor and unique environmental factor. The model fitting of each measurement suggested that periodontal disease was mainly affected by environmental factors, while morphological figures and oral functions were influenced by both genetic and environmental factors.**

KEYWORDS: **geriatric dentistry, genetics, oral function, periodontal disease(s)/periodontitis, caries, twins**

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Introduction

Twin studies comparing monozygotic (MZ) and dizygotic (DZ) twins are considerably valuable in genetic research. MZ twins share 100% of their genes, while DZ twins share only half of their segregating genes on average. The findings of heritability would help to predict and prevent oral conditions and also to recognise therapeutic limits.

Past twin studies that identified genetic components of caries, tooth size and morphology (1, 2) reported a strong genetic component behind the number of filled teeth and a weaker genetic component affecting gingival bleeding. It was concluded that genetic factors contribute to interindividual differences in oral health among young adults. Periodontal disease was previously assessed in US twins and approximately half of all variances were found to have a genetic component (3, 4). In a large twin cohort in Sweden (10 000 pairs), genetic factors were shown to contribute to 14% of variation in tooth loss among women and 39% among men, while heritability estimates of periodontal disease were 39% and 33% for women and men, respectively (5). Overall, the results suggest a more substantial role for genetics.

The majority of these past studies in the field of dentistry involved young and not older twins. Oral problems such as tooth loss, decreased masticatory

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function are highly prevalent among middle-aged people. Therefore, it would be useful to detect the heritability of middle-aged or older people.

Most previous twin studies involved self-reported questionnaires or telephone interviews, which are notoriously limited and unreliable because they only rely on self-judgment and memories (3). Such self-assessment would underestimate rather than overestimate the present oral condition; possible underestimation of the genetic component as a measurement error is subsumed in the environmental component of factors not correlated between twins. Some data, such as morphology of teeth and dental arches, radiographic findings, oral functions and periodontal pocket depth, cannot be accurately collected by self-reports.

Moreover, not only oral disease but also oral function is important in evaluating oral health comprehensively. Occlusal force, masticatory performance and stimulated salivary flow rate (SSFR) are important objective variances of oral function because these variables are directly connected to mastication. In twin studies, the heritability of oral function has never been discussed, although some researchers have reported a genetic predisposition in patterns or levels of physical function (6).

The aim of the study, therefore, was to determine the relative contributions of genetic and environmental factors to oral structures, diseases and functions in middle-aged and old twins.

Materials and methods

Study population

Japanese middle-aged and old twin pairs from all over Japan participated in this cross-sectional study. We carried out the study at the Osaka University Centre for Twin Research (OUCTR), which was founded in 2009 (7). Its original twin registry was launched before 1980 and has expanded to become one of the largest twin registries in Japan (8–10). Eligibility criteria were same-sex, middle-aged and old twin pairs. Written informed consent was obtained from all twins before the clinical examination for which both twins arrived together. The study was independently approved by the Osaka University ethics committee.

Zygosity of the pairs was confirmed using the 15 short tandem repeat (STR) (11) markers derived from blood, which were previously shown to be accurate and reliable (12). A twin pair completely concordant with these STRs was designated MZ. All other pairs were designated DZ.

Dental status and oral function

The dental status of each subject was examined by one of two trained and calibrated periodontists who was blinded to the twin's zygosity. Dental, periodontal and orthopantomographic examinations were conducted to evaluate the dental status. As a screening test for periodontal status, salivary occult blood tested by Perioscreen* was conducted. Probing depth was assessed in six sites (mesiobuccal, mid-buccal, distobuccal, mesiolingual, mid-lingual and distolingual), and the maximum pocket depth of all over sites and teeth was recorded. The bone resorption score was measured using the Schei's ruler technique, in which the percentage of bone loss at the deepest interproximal site of each tooth was measured on an orthopantomograph (13). The total proportion of decayed, filled and missing teeth (DMF) of all teeth (usually 28 teeth except in subjects who had extraction prior to orthodontic treatment or still had wisdom teeth) was used to assess the prevalence of dental caries.

The oral function of each subject was also examined by one of two trained and calibrated prosthodontists. Occlusal force, masticatory performance and SSFR were used to determine the oral function. Occlusal force was measured from a pressure-sensitive sheet (Dental Prescale, 50H-R†). Participants were asked to clench their teeth as hard as possible in the intercuspal position while the pressure-sensitive sheet was placed between their upper and lower dental arches (14). To evaluate masticatory performance, participants were instructed to chew a piece of gummy jelly,‡ a standardised testing food, using 30 chewing strokes on their preferred chewing side and to expectorate the comminuted particles. Masticatory performance was scored by comparing images of visual samples of these particles (15). SSFR was

*Sunster International, Osaka, Japan.

†GC Co., Tokyo, Japan.

‡UHA Mikakuto Co., Osaka, Japan.

collected by the mastication method (16) in which a measured amount of paraffin wax[§] was chewed for 2 min. The distance between the left and right buccal cusps of the first pre-molars for upper and lower plaster models was measured to determine the width of the dental arch.

There were no missing data except for morphologic ones. Alveolar width could not be evaluated in the absence of first pre-molar teeth. In total, 63 individuals had missing units in the upper jaw, while 73 had missing data in the lower jaw. We used the full information maximum likelihood method to handle missing data.

Statistical analysis

In the classical twin study, it is assumed that MZ share 100% of their genes while DZ share 50% of their segregating genes. Based on this assumption, the following three analyses were conducted.

As the first necessary step, we compared means and population variances (the sum of between-pair and within-pair variances) between MZ and DZ twins for each clinical measurement (3). If these values differ between twin groups, estimates of heritability would be biased.

Next, we calculated the similarity of MZ and DZ twins. The value of the intra-class correlation (ICC) could vary from 0 (indicating that the statistical variation within twin pairs was equal to the variation between pairs) to 1 (indicating that all variation is among different twin pairs and there is no variation within pairs).

Finally, we used the quantitative genetic method based on structural equation modelling (17) to estimate genetic and environmental variances and heritability. The comparison between MZ and DZ provides information about genetic and environmental effects on each measurement. Quantitative genetic modelling with the statistical programme Mx (18) permits the estimation of variance components following a comparison of different models (2). For the formal estimation of variance components, we divided phenotypic variance into four latent components: additive genetic effects (*A*), dominant genetic factor (*D*), common environmental effects (*C*) and unique environmental effects (*E*). '*A*' represents all the polygenes whose effects are small and additive to forming a quantitative phenotype. '*D*' denotes the effects of alleles at a locus that do not simply add up to represent genotypic values. '*C*' is the element shared with family members. '*E*' is different from family members even if they live together. The relation between these latent variables and observed variables is depicted in a path diagram as the full model (Fig. 1). This analysis estimates model parameters using maximum likelihood techniques. The goal of this model is to divide the observed variance in phenotype into these four sources. The fits of reduced models were compared with the full model by inspecting changes in χ^2 values relative to differences in the degrees of freedom between models (19). Akaike information criterion [AIC = χ^2 – 2(df)] scores were calculated to provide an additional means of comparing models based on fit and simplicity. Models with the lowest AIC values are generally considered to be the most parsimonious. As the first step, the fully saturated model was compared with the full ACE and ADE model using these variables. If the means and variances are not equal within twins and also between MZ and DZ subjects, the model does not fit. The significance of the genetic variance was tested by inspecting 95% confidence intervals of the A parameter in the full ACE or ADE model, or by examining differences between the AE and E models, adjusted for age and sex using a regression technique.

Results

In this analysis, 132 twin pairs were included. Of these, 116 twin pairs were classified as MZ and 16 as

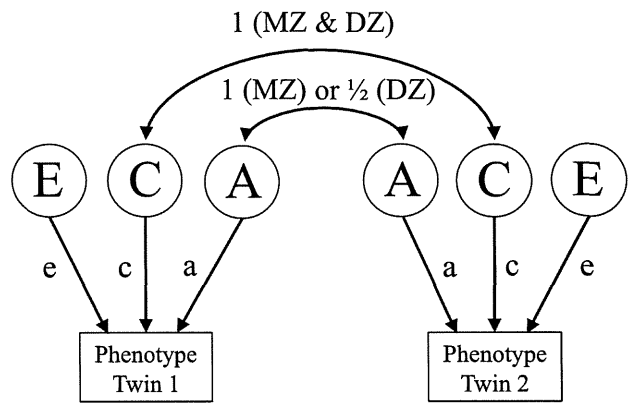

Fig. 1. The path diagram for the basic univariate twin analysis.

[§]Orion Diagnostica, Espoo, Finland.

DZ. The MZ twin pairs consisted of 43 male and 73 female twin pairs, while the DZ twin pairs consisted of eight male and eight female twin pairs. The mean age of all subjects was 66·1 ± 10·3 (SD) years. Salient characteristics of the twins are summarised in Table 1. There was no significant difference between MZ and DZ twins in any clinical parameter. The population variances also did not differ ($P > 0·2$) between the groups for any measure, validating an important assumption of the twin model.

Age- and sex-adjusted ICCs are indicated in Table 2. Although the ICC of three items, occult blood testing of DZ twins and maximum probing depth of MZ and DZ twins were not significant, the remaining 17 items were significant. The ICC with six variables out of ten variables was greater for MZ twins than DZ twins. To further explore heritability, formal genetic modelling was carried out for each variable to determine the best-fitting model (Tables 3 and 4). The best-fit models for the total number of remaining teeth (*C*: 65%, *E*: 35%), occult blood test (*C*: 32%, *E*: 68%) and alveolar bone resorption (*C*: 63%, *E*: 37%) were all CE models. And maximum probing depth was E model. The best-fit model for DMF (*A*: 52%, *E*: 48%) was the AE model.

The best-fit models for occlusal force (*A*: 45%, *E*: 55%) and masticatory performance (*A*: 63%, *E*: 37%) were both AE models. These variables represented masticatory function. SSFR as the variable of oral function (*A*: 51%, *E*: 49%) also fitted in the AE model. The best-fit models for the width of upper dental arch (*A*: 28%, *E*: 72%) and lower dental arch (*A*: 29%, *E*: 71%) were both AE models.

Discussion

The present study indicated the heritability of oral health in the later stages of life. Periodontal disease and number of teeth are influenced by environmental factors. In contrast, morphological figures, dental

Table 1. Clinical and behavioural characteristics of monozygotic (MZ) and dizygotic (DZ) twins

	MZ (n = 232*)			DZ (n = 32*)			
	Mean (SD)	Minimum Maximum	Median	Mean (SD)	Minimum Maximum	Median	*P*-value
Age (years)	65·6 (10·3)	50 88	65	69·8 (14·0)	51 84	69	0·87
Total number of remaining teeth†	21·9 (8·6)	0 32	25	20·9 (9·7)	0 31	25·5	0·77
Occult blood test (0, 1, 2)	0·93 (0·92)	0 2	1	1·22 (0·87)	0 2	1·5	0·73
Maximum probing depth (mm)	5·9 (2·1)	3 12	6·0	6·2 (2·0)	3 12	6·2	0·82
Score of bone resorption	1·9 (0·94)	1 4	1·5	2·0 (1·04)	1 4	1·6	0·82
Percentage of decayed, filled and missing teeth (%)	67·1 (24·6)	3 100	70·9	72·4 (24·9)	20 100	76·8	0·98
Occlusal force (N)	466 (276)	24 1350	423	577 (301)	63 1264	473	0·84
Masticatory performance (mm^2)	3541 (950)	425 5912	3710	3253 (1310)	431 6820	3130	0·54
Stimulated salivary flow rate (ml min^{-1})	1·5 (0·9)	0·1 11·4	1·3	1·3 (0·9)	0·3 (5·3)	1·2	0·99
Width of upper dental arch (mm)	42·9 (3·7)	24 55	43	43·5 (4·1)	36 51	44	0·81
Width of lower dental arch (mm)	35·1 (4·5)	20 42	35	36·7 (2·9)	31 41	36·8	0·45

P-value was calculated by *F* test to compare MZ and DZ observed variables.
*Number of twin individuals.
†Include third molars.

Table 2. Twin intra-class correlation for age- and sex-adjusted clinical measures

	Intra-class correlation (CI)	
	Monozygotic	Dizygotic
Total number of remaining teeth	0·64 (0·52, 0·73)	0·77 (0·46, 0·91)
Occult blood test	0·31 (0·16, 0·45)	0·12 (−0·28, 0·48)
Maximum probing depth	0·10 (−0·12, 0·30)	0·11 (−0·49, 0·65)
Score of bone resorption	0·63 (0·48, 0·74)	0·68 (0·28, 0·88)
Percentage of decayed filled and missing teeth	0·52 (0·38, 0·66)	0·48 (0·34, 0·62)
Occlusal force	0·45 (0·29, 0·59)	0·23 (0·15, 0·30)
Masticatory performance	0·54 (0·38, 0·67)	0·27 (0·20, 0·34)
Stimulated salivary flow rate	0·49 (0·33, 0·62)	0·24 (0·17, 0·31)
Width of upper dental arch	0·28 (0·02, 0·50)	0·14 (0·02, 0·26)
Width of lower dental arch	0·24 (0·02, 0·44)	0·12 (0·01, 0·23)

CI, 95% Confidence Interval.

caries and oral function are affected by both genetic and environmental factors.

Contrary to previous studies (2, 3, 5), our data suggested that genetic factors did not significantly contribute to the number of teeth and periodontal status in older persons. A possible reason for this could be that our participants were relatively older than the subjects of other investigations and were more affected by environmental factors. Furthermore, previous studies primarily considered major oral diseases to be behavioural diseases (20), in which individuals are able to control the extent or onset of disease by adopting a healthy lifestyle including a good diet, oral self-care (21) and regular dental check-ups (22). Age and smoking are also established as important environmental factors affecting periodontal disease (4, 8).

By contrast, we observed that shared environmental factors, which represent familial experiences and habits common within twin pairs, played a significant role in periodontal status and the number of teeth. They accounted for around two-thirds of variability in the number of teeth in middle-aged and old persons. Future studies should identify the shared environmental factors that influence the presence of oral diseases among MZ discordant twins.

The genetic factor was shown to contribute to the presence of dental caries, morphological figures and masticatory functions. The fact that dental caries and not periodontal disease have a genetic contribution may be reflected in the earlier onset of the former. After all, dental caries may be more susceptible to the effect of inheritable immunity function from the bacteria than periodontal disease. Conversely, periodontal disease would be more affected by environmental factors such as oral hygiene practices, smoking, alcohol intake and dietary patterns than dental caries. Morphological figures, which are parts of the skeletal framework of the human body appear to be influenced by genetic factors, similar to the height and weight of an individual (23), while this is also likely for oral function. A possible reason for this is that they are influenced more by anatomical and physiological conditions than pathological changes.

The present study had the following strengths. First, the participants were middle-aged and old twins who were older than those in most twin studies that focused on a younger generation before the onset of severe periodontitis, tooth loss, dry mouth and masticatory disorders. Generally, the oral condition would have changed, and the individual difference would also have begun to widen from middle age.

Second, our data included oral functions objectively measured by highly trained dental professionals. Previous comparisons between self-perceived oral health and clinical findings have shown various degrees of usefulness in determining the number of teeth and the presence of removable prostheses; however, they are less useful in identifying dental caries and periodontal disease (24–26). Additionally, objective oral functions, dental morphology and radiographic findings cannot be measured by self-assessment. The high prevalence of caries and periodontal diseases in old persons reduces the probability of accurate self-assessment, mostly resulting in underestimation. In our study, experienced prosthodontists and periodontists examined the oral status of the participants in person, so our records were highly reliable. However, a major limitation of our study was the sample size, which may affect the generalisability of the findings.

In conclusion, structural equation modelling of middle-aged and old twin pairs suggested that genetic

Table 3. Model fitting result

	−2log likelihood	Difference degree of freedom	Akaike information criterion	*P*
(1) Total number of remaining teeth				
Full saturated	1812	NA	1304	
Full ACE	1817	6	1297	0·55
Full ADE	1822	6	1302	0·12
Full AE	1822	1	1300	<0·01
Full CE	1817	1	1295	0·99
E	1809	2	1366	<0·01
(2) Occult blood test				
Full saturated	631	NA	161·5	
Full ACE	638	6	155·7	0·41
Full ADE	638	6	156·4	0·33
Full AE	638	1	154·4	0·40
Full CE	638	1	153·7	1·00
E	651	2	164·5	<0·01
(3) Maximum probing depth				
Full saturated	886·0878	NA	490·0878	
Full ACE	888·4528	6	480·4528	0·88
Full ADE	888·4523	6	480·4523	0·88
Full AE	888·4528	1	478·4528	0·98
Full CE				
E	889·2907	2	477·2907	0·66
(4) Score of bone resorption				
Full saturated	515·8	NA	115·8	
Full ACE	521·5	6	109·5	0·46
Full ADE	524·4	6	112·4	0·20
Full AE	524·4	1	110·4	0·09
Full CE	521·5	1	107·5	0·96
E	574·1	2	158·1	<0·01
(5) Decayed, filled and missing teeth				
Full saturated	515·8	NA	115·8	
Full ACE	−42·9	40	−523·0	1
Full ADE	−43·4	40	−523·4	1
Full AE	−42·9	1	−525·0	0·54
Full CE				
E	7·8	2	−476·2	<0·01
(6) Occlusal force				
Full saturated	3544·3	NA	3056·3	
Full ACE	3551·5	6	3051·5	0·31
Full ADE	3551·3	6	3051·3	0·32
Full AE	3551·5	1	3049·5	0·68
Full CE				
E	3580·0	2	3075·9	<0·01
(7) Masticatory performance				
Full saturated	2852·3	NA	2388·3	
Full ACE	2862·3	6	2386·3	0·12
Full ADE	2862·5	6	2386·5	0·11
Full AE	2862·5	1	2384·5	0·61
Full CE	2865·4	1	2387·4	0·08
E	2912·3	2	2432·3	<0·01

Table 3. (continued)

	−2log likelihood	Difference degree of freedom	Akaike information criterion	*P*
(8) Stimulated salivary flow rate				
Full saturated	998·9	NA	498·9	
Full ACE	1011·7	6	499·7	0·05
Full ADE	1010·5	6	498·5	0·07
Full AE	1011·8	1	497·7	0·27
Full CE				
E	1046·1	2	530·1	<0·01
(9) Width of upper dental arch				
Full saturated	−703·5	NA	635·8461	
Full ACE	−716·9	6	634·5315	0·10
Full ADE	−620·3	6	634·3749	0·21
Full AE	−620·5	1	632·5315	0·69
Full CE				
E	−620·9	2	638·7	<0·01
(10) Width of lower dental arch				
Full saturated	719·5	NA	463·47	
Full ACE	725·7	6	457·7296	0·40
Full ADE	725·7	6	457·7281	0·40
Full AE	725·7296	1	455·7296	0·97
Full CE				
E	730·7952	2	458·7942	0·08

Table 4. Percentage of variance of additive genetic (*A*), common environmental (*C*) and unique environmental (*E*) variances for mean clinical scores

	Model	*A* (95% CI)	*C* (95% CI)	*E* (95% CI)
Total number of remaining teeth	CE		0·65 (0·46, 0·93)	0·35 (0·28, 0·46)
Occult blood test	CE		0·32 (0·14, 0·68)	0·68 (0·52, 0·88)
Maximum probing depth	E			1·00 (0·83, 1·21)
Score of bone resorption	CE		0·63 (0·43, 0·90)	0·37 (0·28, 0·49)
Decayed, Filled and missing teeth	AE	0·52 (0·38, 0·66)		0·48 (0·34, 0·62)
Occlusal force	AE	0·45 (0·30, 0·60)		0·55 (0·40, 0,70)
Masticatory performance	AE	0·63 (0·54, 0·99)		0·37 (0·22, 0·41)
Stimulated salivary flow rate	AE	0·51 (0·35, 0·63)		0·49 (0·37, 0·65)
Width of upper dental arch	AE	0·28 (0·04, 0·52)		0·72 (0·48, 0·96)
Width of lower dental arch	AE	0·29 (0·02, 0·46)		0·71 (0·54, 0·98)

CI, Confidence Interval.
The structural equation modelling was used, and the fittest model was estimated with Akaike information criteria, log likelihood, degree of freedom and *P*-value.

factors contributed significantly to salivary flow rate, occlusal force and masticatory performance but not to the number of teeth and periodontal status. These findings regarding the genetic contribution, particularly to oral function, would help improve awareness among both dental practitioners and patients and would play an important role in treatment planning.

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Conflicts of interest

The authors declare no potential conflict of interest.

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