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Craniofacial morphology in Chinese female twins: a semi-longitudinal cephalometric study

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SUMMARY It would be of benefit to have a better understanding of the relative effects of genetics and environmental factors on craniofacial parameters when undertaking orthodontic therapy and treatment planning. However, there is a lack of such information in pre-adolescents. The aim of this study was to verify the degree of genetic and environmental contribution to the growth of the facial skeleton in twins aged 6 to 12 years. The material comprised the lateral cephalograms of 89 pairs of female twins in Beijing, China, of whom 61 pairs were diagnosed by DNA analysis as monozygotic (MZ) and 28 pairs as dizygotic (DZ). Four main groups (with a starting age of 6, 7, 9 and 11 years) were studied in a semi-longitudinal manner, with a sub-group further investigated for 2–4 consecutive years. The total sample therefore consisted of 183 pairs (MZ 110, DZ 73) aged from 6 to 12 years. The depths of the cranial base, mid and lower face were measured, as well as anterior and posterior face height.

A two-tailed *t*-test showed significant environmental effects on lower face depth ($P < 0.01$), whilst genetic effects on face height were also significant ($P < 0.01$). The results suggest that early orthodontic intervention would have a greater influence on the antero-posterior rather than on the vertical plane of growth.

Introduction

Orthodontic growth modification has generated controversy in recent years (Gianelly, 1995; White, 1997, 1998; Bowman, 1998; Dugoni, 1998). Twins serve as a unique resource for evaluating the interactions between genetic and environmental effects, helping to provide a more scientifically based rationale for orthodontic treatment (Lauweryns *et al*., 1993).

Many polygenic craniofacial traits are susceptible to environmental modification, and can be difficult to study with conventional methods (Bixler, 2000). Twin studies provide an opportunity to analyse such traits. Classical methods of analysis have been based on comparisons of the differences within pairs of monozygous (MZ or identical) and dizygous (DZ or fraternal) twins, the extent of the differences being taken as an indication of the relative genetic influence on variation of the characteristic studied (Lundström, 1984).

In order to verify the degree of genetic contribution to the growth of the facial skeleton, only a few investigators have undertaken longitudinal observations on a group of twins aged 6–12 years. Dudas and Sassouni (1973) studied 22 pairs of twins, 12 being MZ (10 female and 2 male) and 10 DZ (7 male and 3 female). All twins were examined from 4 to 13 years of age during their growth period. This was the first reported study designed to evaluate twin growth, though limited by the small sample size. However, in view of the large number of variables present, it was difficult to assess the longitudinal effects.

Nakata (1978) made his craniofacial observations over a period of 7 years, starting at 3 years of age. He investigated 28 MZ twins, 9 DZ twins, and 12 twins of unknown zygosity. Unfortunately, due to the small number of DZ twins (three 3-year-old twins, three 4-year-old twins, six 7-year-old twins, six 8-year-old twins, four 11-year-old twins, and four 12-year-old twins) he could not draw any clear conclusions.

Ishikawa (1991) studied 12 MZ and 13 DZ male twins from 6 to 15 years of age utilizing lateral cephalograms. He found that mandibular height showed the lowest heritability, but all the mandibular dimensions seemed to converge to almost the same high range of heritability with increasing age. However, there were a number of limitations to the study design, which related to the accuracy of zygosity determination (using the samples of 30 years ago) and the use of heritability.

The aim of the present investigation was to explore the genetic and environmental influences on craniofacial dimensions in a group of pre-adolescent subjects, using the twin study method.

Materials and methods

The sample consisted of lateral cephalograms of 89 pairs of female twins in Beijing, China. Sixty-one pairs were diagnosed as MZ and 28 pairs as DZ. Zygosity was determined by DNA fingerprinting of the twins using human minisatellite probe 33.15. Based on DNA fingerprints, on

average 32.5 bands were detected and the coincidence of a band in twin sisters was 0.511. Therefore, the probability that sisters of the DZ twins shared an identical DNA fingerprint was 3.34×10^{-10}, calculated as $0.511^{32.5}$, implying an accuracy of 99.99 per cent (Hill and Jeffreys, 1985).

Using a mixed longitudinal method, i.e. further twin pairs were recruited during the trial and added to the original sample of 89 pairs, a total sample size of 183 pairs (MZ 110, DZ 73) of female twins, ranging in age from 6 to 12 years, was analysed (Table 1).

The initial samples were grouped primarily into ages 6, 7, 9 and 11 years and were re-examined the following year. To increase the number of DZ twins, additional DZ pairs, aged from 7 to 10 years, were recruited to the study over three to four consecutive years (see Appendix, Tables A1 and A2).

All procedures were carried out with the informed consent of the parents following protocols reviewed and approved by the appropriate institutional review boards of the Peking University Health Science Centre.

The lateral cephalograms were obtained with a Siemens Orthophos CD system (Pelton & Crane, Charlotte, North Carolina, USA). Each radiograph was traced onto frosted acetate paper and digitized (Microtek, Phantom 3500 Scanner, Zhongjing Computer Company Limited, Shanghai, China) to a computer. Linear dimensional values were measured using computer software (School of Software, Tsinghua University). One hundred and fifty-two radiographs were randomly selected and the tracings and measurements repeated by the same investigator (JP) after an interval of one month to determine measurement errors (ME) in each age group (Table 1).

The landmarks used are shown in Figure 1. Each dimension was measured to the nearest 0.5 mm. Comparisons were carried out between the average intra-pair differences (AID) of ME and MZ twins, MZ and DZ twins, expressed as AID(ME), AID(MZ) and AID(DZ).

Data were analysed using the Statistical Package for Social Sciences (SPSS/PC for Windows, version 10, Chicago, Illinois, USA). In some instances, the variances between the two groups were not found to be the same when applying Levene's test for equality of variances. An F test would therefore have been inappropriate and, for this reason, a *t*-test was used in the analyses. A two-tailed *t*-test was used as it is more conservative than a one-tailed test.

Since MZ twins are assumed to possess identical genetic material, any differences between them must be attributable to environmental factors. Measurement errors were included in the analysis of MZ twins. Therefore, environmental factors are represented by the difference between the average variability of MZ twins and the ME. If the difference between them is significant, then environmental factors are considered to play a role.

The differences in average variability between DZ and MZ twins may be used as an estimate of the genetic

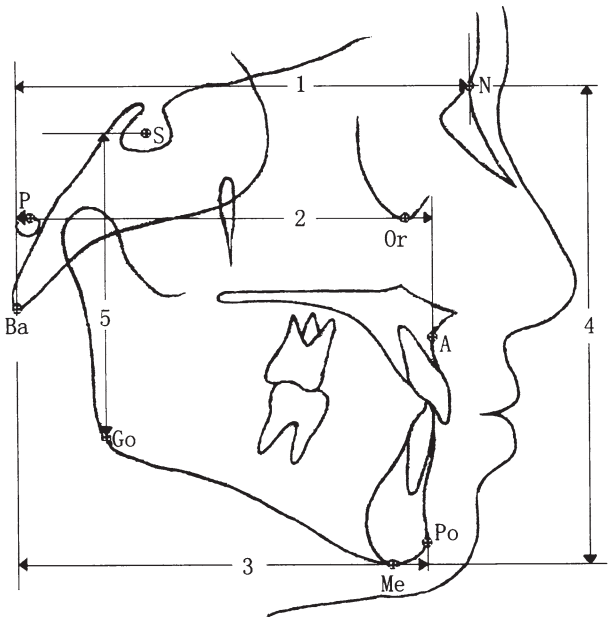

Figure 1 Landmarks and linear dimensions used: anatomic porion (P), orbitale (Or), sella (S), nasion (N), basion (Ba), point A (A), gonion (Go), pogonion (Po) and menton (Me). Three depths [of the cranial base (1), the mid (2) and the lower (3) face] and two heights [of the anterior (4) and posterior face (5)] were included according to the method of Coben (1955).

Table 1 Distribution of measurement errors (ME), and the number of monozygous (MZ) and dizygous (DZ) pairs used in the study.

Age (years)	ME	MZ			DZ		
	n (pairs)	*n* (pairs)	Mean age (months)	SD (months)	*n* (pairs)	Mean age (months)	SD (months)
6	8	7	76.1	1.3			
7	20	17	85.7	3.8	8	84.9	4.3
8	22	12	96.5	4.2	9	96.8	4.0
9	31	16	107.6	3.4	13	106.6	3.7
10	30	19	119.1	3.5	15	118.9	3.9
11	22	22	130.6	3.1	14	131.8	3.3
12	19	17	143.2	3.2	14	143.2	3.5

SD, standard deviation.

portion of total variation. Again, variability between MZ may be due to environmental influences and ME. When twins are homologous (same race, same geographical area, etc.), the average environmental differences for DZ and MZ twins are the same. Homogenity of the MZ and DZ types must be statistically tested (Christian, 1979; Sharma and Corruccini, 1986). Most differences in means between zygosity groups were not significant in the present study. Even if there were statistical differences, these were very small (Peng, 2002). Therefore, the differences between average variability of DZ and MZ twins can be attributed to genetics. If the difference between DZ and MZ twins is significant, then a genetic source of variation is considered to be present.

Results

Cranial base depth

The significant difference between AID(MZ) and AID(ME) indicated that measurement of cranial base depth was sufficiently accurate to detect environmental influences affecting MZ twins at 9, 11 and 12 years of age (Table 2). Based on a comparison of AID(MZ) and AID(DZ), a genetic contribution to variation was detectable at 8 years of age (Table 3).

Mid face depth

A similarly significant difference between AID(MZ) and AID(ME) was found at 8, 9, 11 and 12 years of age (Table 2). Comparison of the difference between AID(MZ) and AID(DZ) revealed a genetic contribution to the variation at 10 years of age (Table 3). In addition, the data indicated that mid face depth was more sensitive to environmental effects than cranial base depth.

Lower face depth

The difference between AID(MZ) and AID(ME) was statistically significant at 8, 9, 10, 11 and 12 years of age (Table 2). Based on comparison of AID(MZ) and AID(DZ), a genetic contribution to variation was detectable only at 10 years of age (Table 3). Environmental influences provided the greatest contribution to variability of lower face depth among the observed dimensions.

Anterior face height

The significant difference between AID(MZ) and AID(ME) showed that measurement of anterior face height was sufficiently accurate to detect environmental influences producing differences between MZ twins at 7 and 9 years of age (Table 2). However, a clear genetic contribution to variation was apparent at 9, 10, 11 and 12 years (Table 3).

Posterior face height

In addition to significant differences between AID(MZ) and AID(ME) at 7, 9, 10, 11 and 12 years of age (Table 2), there were also statistically significant differences between AID(MZ) and AID(DZ) at 8, 10, 11 and 12 years of age (Table 3).

Table 2 Mean intra-pair differences in linear dimensions of monozygous (MZ) and measurement error (ME) pairs (independent-sample *t*-test).

Linear measurement	Aged 6 years		Aged 7 years		Aged 8 years		Aged 9 years		Aged 10 years		Aged 11 years		Aged 12 years	
	Variance	*P*	Variance	*P*	Variance	*P*	Variance	*P*	Variance	*P*	Variance	*P*	Variance	*P*
Cranial base depth														
MZ	1.3		1.7		1.6		2.3		1.7		1.6		1.9	
ME	1.0	0.484	1.2	0.320	1.0	0.122	0.6	0.000	1.0	0.089	0.8	0.031	0.7	0.023
Middle face depth														
MZ	1.3		1.5		1.9		1.7		1.6		2.1		2.7	
ME	0.6	0.157	0.9	0.269	0.8	0.027	0.6	0.024	0.9	0.068	0.8	0.001	0.6	0.004
Lower face depth														
MZ	1.7		2.1		2.9		2.8		2.7		2.9		4.0	
ME	0.6	0.137	1.1	0.073	0.9	0.035	0.7	0.005	0.9	0.007	0.7	0.002	1.1	0.023
Anterior face height														
MZ	1.3		2.1		1.2		2.3		2.2		2.0		2.1	
ME	1.0	0.355	1.0	0.033	1.0	0.509	1.2	0.005	1.6	0.156	1.4	0.245	1.4	0.194
Posterior face height														
MZ	0.6		1.8		1.1		1.9		1.9		1.7		1.9	
ME	0.4	0.495	0.7	0.011	0.8	0.180	0.7	0.015	0.6	0.001	0.6	0.000	0.8	0.009

Note: Levene's test for equality of variances was carried out.

Table 3 Mean intra-pair differences in linear dimensions of monozygous (MZ) and dizygous (DZ) twins (independent-sample *t*-test).

Linear measurement	Aged 7 years		Aged 8 years		Aged 9 years		Aged 10 years		Aged 11 years		Aged 12 years	
	Variance	*P*	Variance	*P*	Variance	*P*	Variance	*P*	Variance	*P*	Variance	*P*
Cranial base depth												
MZ	1.7		1.6		2.3		1.7		1.6		1.9	
DZ	2.9	0.138	3.1	0.037	2.8	0.382	2.5	0.147	2.0	0.467	2.4	0.380
Middle face depth												
MZ	1.5		1.9		1.7		1.6		2.1		2.7	
DZ	2.6	0.057	2.6	0.345	2.6	0.162	3.4	0.009	2.5	0.566	2.6	0.951
Lower face depth												
MZ	2.1		2.9		2.8		2.7		2.9		4.0	
DZ	2.6	0.567	4.9	0.160	3.9	0.243	5.1	0.022	3.8	0.351	3.6	0.800
Anterior face height												
MZ	2.1		1.2		2.3		2.2		2.0		2.1	
DZ	3.5	0.250	3.5	0.055	5.6	0.042	6.7	0.001	6.3	0.002	5.7	0.001
Posterior face height												
MZ	1.8		1.1		1.9		1.9		1.7		1.9	
DZ	2.4	0.338	3.5	0.026	3.1	0.131	4.0	0.014	4.4	0.001	4.6	0.024

Note: Levene's test for equality of variances was carried out.

Discussion

For over 100 years, twin studies have served as a basic tool in evaluating the relative contribution of genetic and environmental factors (Corruccini *et al*., 1990; Harris and Potter, 1997). However, the twin method is limited in several ways, not only because it is difficult to obtain a sufficient number of twin pairs, but also because it can be difficult to establish zygosity and confirm that environmental factors are in fact the same for both members of a twin pair (Proffit, 2000).

In this investigation, 183 pairs of female MZ and DZ twins ranging in age from 6 to 12 years and demonstrating good homogenity (same race, same geographical area, etc.) were studied. This was confirmed by the homogenity of the twin types and the similar measurement means for both groups (Christian, 1979; Sharma and Corruccini, 1986). Even when statistical differences between the twin groups were detected, they were very small (Table 3). For these reasons, comparisons of the MZ and DZ types were likely to be unbiased. Moreover, zygosity was confirmed by DNA fingerprinting with an accuracy of 99.99 per cent (Hill and Jeffreys, 1985).

Heritability has been calculated in the majority of twin studies, but for some investigations the closest approach to heritability is the proportion of variance between DZ pairs that is lost when the genotype is held constant (Osborne and DeGeorge, 1959). There are some other shortcomings in determining heritability, such as bias caused by measurement accuracy and the method of statistical analysis applied (Shapiro, 1969; Harris and Potter, 1997).

The high significance of AID(MZ) and AID(ME) suggests that the measurements were sufficiently accurate to permit detection of environmentally influenced differences between MZ twin pairs. Significance of the MZ and DZ comparisons suggests a relatively large genetic component of variability in a population for a trait. The present approach is less misleading than heritability and, more importantly, lends itself to tests of significance.

Based on the statistical results (Table 2), environmental influences were observed for the three measurements of facial depth. When the level of significance and also the AID are considered, lower face depth appeared to be most sensitive to these environmental effects. In spite of the detection of environmentally influenced differences between MZ twin pairs, the AID of MZ (Table 3) were relatively low, suggesting that the genotype may be held constant, especially for cranial base and mid face depths. In addition, the comparisons of AID(MZ) and AID(DZ) for the three facial depths were not significant for most age groups (Table 3), indicating two effects: either that the genotype is held constant, or that the phenotype is sensitive to environmental influence. Further analysis suggests that cranial base depth tends to be genetically constant, lower face depth tends to be environmentally influenced, and mid face depth lies between the two.

Anterior face height was less significant for AID(MZ) and AID(ME), but it was significant for AID(MZ) and AID(DZ), indicating a relatively large genetic component of variability. It is of interest that posterior face height was significant for AID(MZ) and AID(ME) and also for AID(MZ) and AID(DZ), which suggests that this variable

has detectable environmental effects but may also be genetically influenced.

It should be noted that there was no clear trend observed in relation to the age groups for the different variables. This may be a sampling problem or may indicate significant genetic input at only certain ages. A possible explanation for some of these inconsistencies might be differences in the timing of onset, velocity and duration of adolescent growth spurts that at least some of the twins had probably entered. Again, with a relatively small sample and a mixed longitudinal study design, individual differences in the growth pattern within and between pairs may to some extent cause inconsistencies in the final results. Moreover, given that the information in Table 2 essentially indicates whether the variation between MZ pairs exceeds that due to ME, it could be argued that only those variables that show significant differences in Table 2 should be considered in Table 3. If this theory is followed, the evidence for significant genetic effects on craniofacial variables is somewhat reduced. Fortunately, the ME in the study was very small.

In order to verify the degree of genetic contribution to the growth of the facial skeleton, investigators have made longitudinal observations on groups of twins aged 6 to12 years (Dudas and Sassouni, 1973; Nakata, 1978; Ishikawa, 1991). In the absence of a larger sample size, clear conclusions cannot be drawn from these studies.

Although a recent cross-sectional twin study of 10 to 13 year olds (Manfredi *et al.*, 1997) was somewhat controversial, i.e. using heritability for analysis and including different genders in the twin samples (Harris and Potter, 1997), there were several intriguing observations relevant to the present research. These cross-sectional adolescent and adult twin studies reported the same findings related to facial depth and height (Horowitz *et al*., 1960; Hunter, 1965) which suggests that there might be a 'universal influence' of genetic and environmental factors in craniofacial dimensions for both children and adults.

Even if craniofacial dimensions have relatively high inherited proportions, the environmental influences on craniofacial morphology should not be ignored in orthodontic treatment planning. Various parts of the craniofacial morphology respond differently to different environmental influences. Horizontal factors decline in genetic contribution as age progresses towards 12 years, whilst vertical factors show the opposite trend. These findings may help clinicians in early orthodontic treatment planning by providing them with a better overall understanding of the factors contributing to variation. If early orthodontic treatment is to be effective, perhaps intervention in the antero-posterior plane would offer improved long-term stability, whilst the vertical plane should perhaps be left to develop into the adult phenotype before treatment. This indicates that the majority of treatment effects should be directed in the antero-posterior rather than the vertical plane, especially for lower face depth.

Furthermore, it should be noted that although vertical factors appear to be strongly genetically influenced during the later stages of development, growth modification may be undertaken prior to this. Twin studies merely quantify the extent of genetic and environmental contributions to observed variation in the group under investigation at a given time. The genetic and environmental estimates are not mutually exclusive of each other and they should not be applied directly to an individual in deciding whether a particular treatment will or will not be successful.

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Appendix

Table A1 Increased monozygous (MZ) and dizygous (DZ) twin samples in each of the four consecutive observation years.

	6 years	7 years		8 years		9 years		10 years		11 years		12 years	
	MZ	MZ	DZ	MZ	DZ	MZ	DZ	MZ	DZ	MZ	DZ	MZ	DZ
First year	7	11	8	2	1	14	8	7	2	17	6	3	2
Second year	0	6	0	10	8	2	1	12	9	5	2	14	6
Third year	0	0	0	0	0	0	4	0	1	0	5	0	1
Fourth year	0	0	0	0	0	0	0	0	3	0	1	0	5
Total pairs	7	17	8	12	9	16	13	19	15	22	14	17	14

Table A2 Serial numbers of monozygous (MZ) and dizygous (DZ) twin pairs in each age group (6 to 12 years).

6 years MZ	7 years MZ	7 years DZ	8 years MZ	8 years DZ	9 years MZ	9 years DZ	10 years MZ	10 years DZ	11 years MZ	11 years DZ	12 years MZ	12 years DZ
125, 126	9, 10	1, 2	3, 4	115, 116	3, 4	115, 116	7, 8	93, 94	35, 36	93, 94	29, 30	47, 48
131, 132	13, 14	11, 12	9, 10	1, 2	7, 8	17, 18	27, 28	115, 116	41, 42	115, 116	45, 46	93, 94
133, 134	37, 38	19, 20	37, 38	11, 12	27, 28	23, 24	31, 32	17, 18	51, 52	25, 26	181, 182	25, 26
145, 146	53, 54	81, 82	53, 54	19, 20	31, 32	69, 70	43, 44	23, 24	57, 58	63, 64	21, 22	63, 64
147, 148	61, 62	109, 110	83, 84	81, 82	43, 44	155, 156	75, 76	69, 70	113, 114	67, 68	39, 40	67, 68
61, 62	83, 84	117, 118	95, 96	109, 110	75, 76	157, 159	77, 78	155, 156	21, 22	79, 80	59, 60	79, 80
143, 144	95, 96	127, 128	105, 106	117, 118	77, 78	161, 162	89, 90	157, 159	39, 40	103, 104	71, 72	103, 104
	105, 106	135, 136	123, 124	127, 128	89, 90	1, 2	101, 102	161, 162	59, 60	195, 196	85, 86	195, 196
	123, 124		129, 130	135, 136	101, 102	11, 12	149, 150	173, 174	71, 72	157, 158	87, 88	157, 158
	129, 130		139, 140		149, 150	81, 82	153, 154	1, 2	85, 86	158, 159	91, 92	158, 159
	139, 140		141, 142		153, 154	109, 110	163, 164	81, 82	87, 88	157, 159	97, 98	157, 159
	141, 142		165, 166		163, 164	157, 158	167, 168	109, 110	91, 92	17, 18	107, 108	17, 18
	143, 144				167, 168	158, 159	187, 188	157, 158	97, 98	23, 24	111, 112	23, 24
	125, 126				169, 170		205, 206	158, 159	107, 108	199, 200	119, 120	73, 74
	131, 132				171, 172		35, 36	199, 200	111, 112		98, 99	
	133, 134				165, 166		41, 42		119, 120		97, 99	
	147, 148						51, 52		175, 176		203, 204	
							57, 58		185, 186			
							113, 114		203, 204			
									98, 99			
									97, 99			
									201, 202			
*n*7	17	8	12	9	16	13	19	15	22	14	17	14

Note: 97, 98 and 99 were triplets with the same DNA fingerprinting. 157, 158 and 159 were triplets whose DNA fingerprinting differed.