Lateral Cephalic Radiograms As An Aid To The Determination Of Monozygosity

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Introduction

It is generally agreed that identical twins are the products of division of an egg which was fertilized by a single spermatozoon, hence the term monozygotic (MZ) twins. Fraternal, or dizygotic (DZ) twins, in contrast, are the products of the simultaneous fertilization of two separate eggs by two different spermatozoa. Monozygotic twins are thus of the same genotype, whereas dizygotic twins are no more alike, save for being the same age, than are any siblings.

Inasmuch as MZ twins are of the same genetic composition, any differences between them (other than the expected mirror-imaging in some instances) are to be attributed to the effects of environment. Identical twins are naturally-occurring experiments which allow direct testing of the interactions of genetics and environment and have been widely used in studies in human genetics (Galton[4], Newman[8], et al., Gedda[5]).

The present study is of this type. Material available for this study was twenty-nine pairs of lateral cephalic radiograms from a mixed sample of twenty-nine pairs of like-sexed MZ and DZ twins, the classification being unknown to the author. The diagnosis of monozygosity and dizygosity had already been made at the Institute of Human Biology, University of Michigan, but purposely this information was not requested until after the preliminary study of the tracings of the radiograms was finished. Thus, the first part of the present investigation was a test of the validity of differentiating between MZ and DZ twins solely on the basis of a study conducted on lateral cephalic radiograms.

This is the first study to use such cephalograms as an aid in diagnosing MZ and DZ twins. Wylie [12], in a widely quoted report, also used lateral cephalograms to measure similarities of angular relationships between cranial base and facial points of parents and children, and between siblings. He studied members of thirteen families, which included some twin pairs, but he did not distinguish between MZ and DZ pairs. With respect to the twins he concluded that those showing pronounced outward similiarity may show dissimilarity in the craniofacial complex.

Kraus[6], et al., using identical and nonidentical triplets as observational material, arrived at essentially the same conclusion with regard to the identical sets, holding that the genes strongly control the various contours of individual bones but that environment is preeminent in determining the "various inter and intrarelationships which together make up a harmonious (or unharmonious) head and face . . . ."

It will be shown that it is possible, with reasonable accuracy, to differentiate monozygous and dizygous twin pairs solely on the basis of tracings of lateral cephalic radiograms. This leads to the obvious inference that inter and intrarelationships of craniofacial parts are

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strongly determined by genetic factors and only slightly modified by effects of environment.

This view is supported by Lundstrom[7] who analyzed the relative effect of genetics and environmental factors on craniofacial relationships. He stated "The genetic factors seem to be consistently more important than the sum of the environmental factors".

Material And Methods

The material for study consisted of craniofacial tracings of midline structures and right and left sides of bilateral structures made on matte acetate of twenty-nine pairs of lateral cephalic radiograms. The radiograms were made according to the Broadbent-Bolton technique in the Department of Orthodontics of the College of Dentistry, University of Michigan. The sets of twins were diagnosed for monozygosity or dizygosity at the Institute of Human Biology, University of Michigan, on the basis of likenesses or dissimilarities of the following serological characters: ABO, MN, Rh (5 sera), K, Fy, and Secretor. Twins concordant for the serological characters were given additional study (kodachrome of iris, and finger and palm prints). On the basis of available data the investigators at the University of Michigan estimated that 97% of the twenty-nine pairs were correctly diagnosed without chance of error (J. N. Spuhler, personal communication).

On each of the tracings the bilateral structures were bisected to render hypothetical midline constructs and the usual points and lines generally employed in cephalometric roentgenology were located (Figs. 1,2).

The tracings of the radiograms were analyzed both for craniofacial relationships (angular measurements) and for magnitudes (linear measurements). For the angular relationships the three

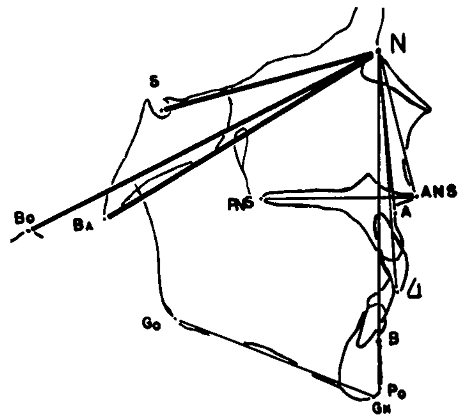

Fig. 1 Angular measurements (several angular measurements are omitted from this illustration).

basicranial lines used, viz.: SN, BoN, BaN, are the standard basicranial reference lines for almost all orthodontic and anthropologic research, and the four points involved can be determined with reasonable certainty. The facial points were selected for their ease of location and for the fact that they are commonly used in studies of this kind. These points and the angles they form with the several basicranial lines define the face in norma lateralis.

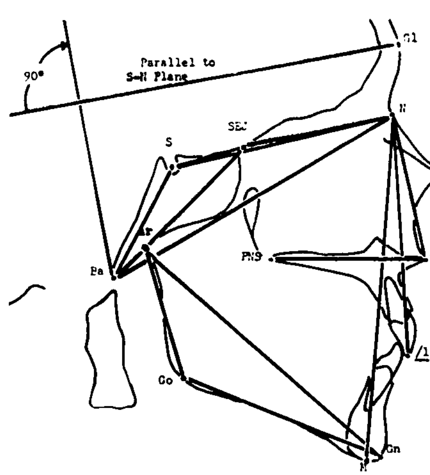

Fig. 2 Linear measurements.

Save for additional points used to measure cranial length and height and one additional point (SEJ) on the cranial base, the linear measurements were made between points selected for the angular measurements.

The thirty-seven measured were:

1) Angles with their apex at N, formed by the intersection of the three basicranial lines with the six facial lines N-A, N-ANS, N-/1, N-B, N-Po, N-Gn.

2) Angles between lines Go-Gn, occlusal line, ANS-PNS, each projected posteriorly to intersect each of the three basicranial reference lines.

3) Cranial base angles, Ba-S-N ("saddle") and Ar-S-N (after Bjork).

4) Angles having Go-Gn line in common, with Go at the apex and the third point at S, Bo, and Ba.

5) Angles from apices S, Bo, Ba, to N and Go.

6) Gonial and BaNS.

The sixteen linear measurements were:

1) Measurements from point N to Ba, S, SEJ, ANS, /1, Mn.

2) Measurements from Ba to S, SEJ, Ptm, Ar, vertex (cranial height).

3) Measurements from Ar to Go and Gn.

4) ANS-PNS, Go-Gn and cranial length.

In a previous work the author[9] sought to determine whether there were certain angles in the craniofacial complex that were more highly predictive of monozygosity than others. This was studied as follows.

All possible angles that could be derived from the lateral head film were measured on a sample of twins of both sexes. They had been classified as to mono or dizygosity at the University of Michigan but their information was withheld from the author at his request.

Each angle was studied for its range of variation within the entire population of the sample and the five angles yielding the highest variation and lowest mean intrapair differences were selected for the testing of the method. It was felt that if angles lying at the limits of the ranges were selected the chance that such an angle would be duplicated in any other individual save an identical twin would be remote. That identity of several angles would be found in two individuals other than such twins seemed improbable.

These five angles were BoN-GoGn, BoN-Po, Ba-S-N, BoN-/1, and BaN-A. The twin pairs which had small intrapair differences in these five angles were called MZ twins, whereas those twin pairs with high intrapair differences were called DZ twins. Twin pairs whose intrapair differences fell between these two extremes were given additional study by superposing the tracings of the individuals of each such pair, and the degree of concordance in entire facial morphology as well as individual parts was noted. An impression could be gained as to whether there was enough similarity to call a twin pair under study monozygous, and they were diagnosed by this impression (Figs 3, 4).

On the basis of this method, fourteen pairs were identified as identical and fifteen were not. When comparison of these findings was made with those derived at the University of Michigan, disagreement was found on just two pairs. One of these was in the monozygotic group, the other in the dizygotic.

In an effort to reconcile these differences recourse was had to a superpositioning of the x-ray tracings. These

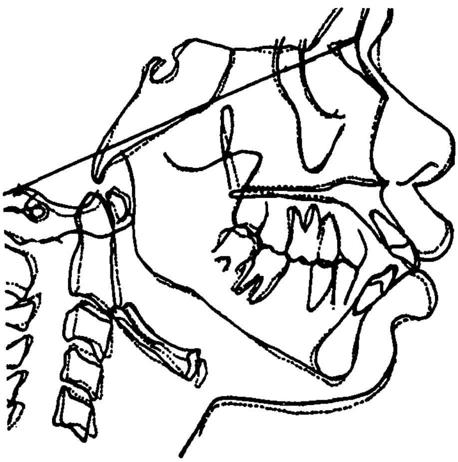

Fig. 3 Monozygous twin pair. Solid line, one twin; dotted line, is co-twin. Both individuals are missing lower first molars, have a foramen for the vertebral artery in the first cervical vertebra and show remarkable concordance throughout.

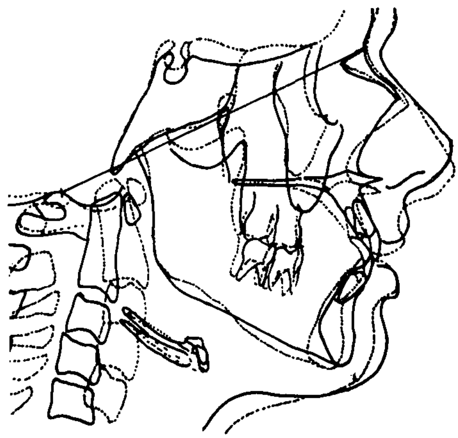

Fig. 4 A dizygous twin pair. Note differences in mandibular position and configuration as well as lack of close conformity in general. Solid line is one twin and dotted line is co-twin.

did not lead to a change in the author's classification because the superposing of additional structures gave strong evidence of identity in one pair. However, the twin and co-twin of the pair classified by the author as identical and by the University of Michigan as nonidentical were remarkably similar when compared by this method.

The present work represents an effort to increase the precision and reproducibility of the x-ray method of appraisal by inspection. Since the classification made at the University of Michigan was based on a greater number of discrete characters (blood types, iris color, finger prints, etc.) than that of the author, it was decided to use that determination as the basis for this aspect of the study. Briefly, our effort was to determine whether reliability would be increased by including more factors such as additional angles and linear dimensions.

A statistical study was made of seven sets of measurements (described in the findings below and in Table I) which were made from the tracings of the radiograms as described earlier. Fifty-three measurements were made on each individual and a twin intrapair difference was calculated for each of these measurements. The intrapair differences were measures of similarity, the smaller the intrapair difference, the greater the similarity. The sum of the intrapair differences for each twin pair was calculated for each of the seven sets of measurements studied for both the MZ and DZ twin groups, and means and standard deviations were calculated for these intrapair differences. Differences between MZ and DZ twin groups were analyzed (Table I) with the unpaired "t" test ("t" test discussed in Batson[1]).

In addition to the differences between the MZ twin group and the DZ twin group, each twin pair was classified as monozygous or dizygous solely on the basis of *each* of the seven sets of measurements. This was done by comparing the sum of intrapair differences of each pair, for a given set of measurements, with the statistics of the

TABLE I

TABLE OF "t" TESTS

GROUPS		MEANS OF STANDARD DEVIATION				"t"
		MZ PAIRS (14 pairs)		DZ PAIRS (15 pairs)		
		Mean	Std. d.	Mean	Std. d.	
LINEAR		37.98	10.10	55.68	15.59	3.65
ANGULAR	BaN line	26.38	9.01	50.10	19.99	4.16
	BoN line	23.19	8.48	44.67	16.65	4.42
	SN line	24.39	9.25	44.54	16.37	4.89
	ALL ANGLES	84.63	21.66	156.97	47.22	5.36
	SELECTED ANGLES	38.51	16.11	75.15	22.61	5.42
TOTAL ALL LINEAR AND ANGULAR		122.61	22.42	212.65	57.13	5.66

TABLE II

TABLE OF OVERLAP
29 LIKE-SEXED TWIN PAIRS

MEASUREMENT GROUP		NUMBER OF INCORRECTLY CLASSIFIED PAIRS		PERCENT ERROR
		MZ	DZ	
LINEAR		0	9	31.0
ANGULAR	BaN line	0	6	20.7
	BoN line	0	9	31.0
	SN line	1	7	27.6
	37 angles	1	3	13.8
	15 selected	0	4	13.8
LINEAR PLUS ANGULAR		0	3	10.3

MZ twin pair group for the same set of measurements. Those twin pairs with intrapair differences of less than two standard deviations of the MZ twin pair group mean were classified monozygous, and those twin pairs with intrapair differences greater than two standard deviations of the MZ twin pair group mean were classified dizygous. This information was tabulated in the form of a "table of overlap" (Table II) which compares the results obtained with each of the seven sets of measurements when these are compared with the University of Michigan's classification of these twin pairs, and gives in percentage the error in differentiating MZ and DZ twin pairs with each of the seven sets of measurements.

Findings

Linear Measurements

Sixteen measurements comprise this group of measurements. The "t" value (3.65) indicates a significant difference between the MZ and DZ twin groups. However, on the basis of the amount of overlap nine DZ pairs fell within two standard deviations of the MZ twin group mean, yielding a 31% error in differentiating MZ and DZ twin pairs.

Angular Measurements

BaN line

Eleven angles were included in this group, all measured with the BaN line as the basicranial reference. The "t" value (4.16) is slightly more significant than the linear measurements. On an individual basis the intrapair differences of six DZ pairs fell within two standard deviations of the MZ twin group mean, yielding a 20.6% error in differentiating MZ and DZ twin pairs.

BoN line

Eleven angles were included in this group, using BoN line as the basicranial reference. The "t" value (4.42) was higher than the equivalent measurements made using BaN line; however, nine DZ twin pairs had intrapair differences less than two standard deviations of the MZ twin group mean, yielding a 31% error in differentiating MZ and DZ twin pairs.

SN line

Eleven angles were included in this group of measurements using the SN line as the basicranial reference. The "t" value (4.89) was higher than any of the previous groups of measurements. Intrapair differences of seven DZ twin pairs fell within, and one MZ twin pair fell outside the two standard deviations of the MZ twin group mean, yielding 27.6% error in differentiating MZ and DZ twin pairs.

All Angular Measurements

Thirty-seven angles comprise this group of measurements, the thirty-three mentioned above (eleven for each basicranial reference line) plus the following four angles, BaNS, ArSN, BaSN and Gonial. This yielded a more significant "t" value (5.36). Intrapair differences of three DZ twin pairs fell within, and one MZ twin pair fell outside the two standard deviations of the MZ twin group mean, yielding a 13.8% error.

Selected Angles

Fifteen angles were selected on the basis of an index which was devised to lessen chance occurrence of small intrapair differences. It was calculated as follows for each of the thirty-seven angles studied:

$$\text{Index of chance error} = \frac{\text{Mean Ang. intrapair diff. (all pairs)}}{\text{Variability (angle, pop. as a whole)*}}$$

*Variability is the square of the standard deviation, by definition. An estimate of the population as a whole was obtained by choosing at random one member of each twin pair, 29 individuals in all.

The index decreases as variability increases and intrapair differences decrease. The angular relationships with small intrapair differences have the probable additional property of possessing a strong genetic determination. This group had a "t" (5.42) only slightly higher than the previous group ("t" 5.36). Four DZ twin pairs had intrapair differences less than two standard deviations of the MZ twin group mean, yielding a 13.8% error.

Linear and Angular Measurements Combined

This group combines all the linear and angular measurements studied, 53 in number; 37 angular, and 16 linear. The highest "t" value (5.66) and the lowest overlap were recorded for this group of measurements. Intrapair differences of three DZ twin pairs were less than two standard deviations of the MZ twin group mean, yielding a 10.3% error in differentiating MZ and DZ twin pairs.

Discussion

Angular Measurements

Comparing SN, BoN, and BaN basicranial reference lines (Table I) indicates "t" values of the same order of significance. There is more overlap between MZ and DZ twin pairs using BoN line (9 pairs) than either SN line (7 pairs) or BaN line (6 pairs). The differences in either case are not remarkable.

Comparing the "selected angles" group with the thirty-seven angle group indicates a "t" value which is slightly greater for the "selected angle" group, but the degree of overlap was the same in both instances.

Linear Measurements

The lowest significant difference between the MZ and DZ twin groups is in linear dimensions. When linear and angular measurements are compared, it is evident that angular intrapair differences in MZ twins are less closely related to those of the DZ twin pairs ("t" 5.36) than are the equivalent linear differences ("t" 3.65). This suggests that linear measurements (size of parts) are more strongly influenced by the randomizing effect of environment, while the angular measurements (relationships of parts) are more strongly influenced by the genetic identity of the MZ twins and the genetic nonidentity of the dizygotic twins.

Linear Plus Angular Measurements

When all the measurements are combined, linear and angular, the difference between MZ and DZ twin pair groups is most marked ("t" 5.66), and the degree of overlap (3 pairs) is less than with any other combination of measurements studied. The identity of monozygous twins is more strongly indicated by *increasing* the number of measurements.

Intrapair Differences

The means of the MZ twin intrapair differences are approximately one-half those of the DZ twin intrapair differences (Table I). Furthermore the standard deviations of these means show that the MZ intrapair differences are tightly clustered about the mean value. These observations clearly establish that the measured angles are closely similar in genetically identical twins, but less similar in genetically nonidentical twins. An obvious inference from such data is that genetic identity is expressed in craniofacial pattern in spite of the randomizing influence of environment.

Lundstrom's[7] findings support this impression. He analyzed the variance of several craniofacial measurements, linear and angular, using MZ and DZ twin pairs as observational material, and found the variability twice as great in DZ twin pairs than in MZ twin pairs. He did not discuss the mean values of the measurements of these twin groups.

Validity of the Differentiating MZ and DZ Twin Pairs

Differentiating the MZ from DZ twin pairs by inspection of the tracings gave the closest compliance to the University of Michigan diagnosis of these twin pairs (27 correct out of 29 pairs). The equivalent procedure on the basis of measurement (26 correct out of 29 pairs) should be more reproducible in different laboratories and clinics and, although less accurate in this study, its statistical significances can be assessed. There is no way to detect error in the University of Michigan diagnoses, if indeed there is any in the twenty-nine pairs studied. It would, of course, be useful to have the blood types of the individuals. Conceivably, some of the twin pairs that contributed to the overlap would have been eliminated if members of such a pair had dissimilar blood types, an obvious indication of dizygosity. It seems ironic that the very pairs of like-sexed twins which would yield the most information, namely the "not-so-similar" MZ twins and the "very similar" DZ twins, still remain the most difficult to diagnose. An absolute diagnosis of MZ and DZ twin pairs may not be possible (Race[10] and Sawyer), but the diagnosis becomes more precise as indices are added which are calculated to minimize the inclusion of DZ pairs with similarities approaching those of MZ pairs. As the number of reliable indices is increased, the probability of a dizygous pair of twins to possess the same degree of similarity as the monozygous twins on the basis of chance alone becomes more and more improbable (Smith and Penrose[11]).

Conclusion

A diagnostic index using the precision of measurement possible on a tracing of a properly oriented cephalic radiograph should increase the accuracy of the diagnosis of monozygosity. In the present study differentiating MZ and DZ twin pairs was accomplished with an error of only 10.3%; consideration of other diagnostic procedures in conjunction with the cephalometric data could reduce the error substantially.

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