

Twins with Clefts: A Descriptive Statistical Analysis of Selected Variables

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To many investigators interested in the etiology of congenital malformations twins are an intriguing source for observations regarding the relative contributions of genetic and non-genetic factors. Twins are sometimes considered to be a type of natural experiment, a compensation to the investigator of human biological phenomena for not being able to make controlled experimental observations on his subjects. In this sense monozygotic (MZ) twins who share 100% of their genes, having developed from the same fertilized ovum, are compared with dizygotic (DZ) twins who, having developed from two separately fertilized ova, share only 50% of their genes (the same as ordinary siblings). Both members of either type of twins, MZ or DZ, are assumed to share a common prenatal environment. This characterization of twin studies is grossly oversimplified, and any investigator interested in twin studies should become familiar with the many methodological limitations which have been well-described by experts (1, 2, 10, 12).

One major limitation of studying relatively rare biological phenomena, such as cleft lip and palate, in twins is the difficulty in acquiring an unselected series of cases large enough to yield reliable results. To illustrate the problem, approximately one in every 50 babies born alive in the United States is a twin. About one in every 800 babies is reported to have a cleft of the lip or palate, though the actual incidence is doubtlessly higher. Thus, it follows that on the average one would have to observe approximately 40,000 births in order to detect one twin with a cleft, assuming that clefting and twinning are independent phenomena.

Through the National Cleft Lip and Palate Intelligence Service, a large, unselected series of birth certificates of twins with congenital malformations and of their co-twins is available for analysis. This series is large in the sense that it is composed of approximately 2,000 certificates of twins with malformations of any kind, 214 of which are clefts. It is unselected in the sense that all certificates of twins in a population of 96,000 birth certificates that listed malformations of any kind are included. In addi-

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tion to certificates mentioning malformations, this series includes a control group of certificates without malformations. The methods involved in the collection of these certificates have been described in a previous publication (6).

The present report is a description of incidence, sex ratios, concordance, and associated malformations among twins with clefts reported on certificates of live birth. Similar data on some other relatively common congenital malformations have been published elsewhere (8). Although in most twin studies the critical comparison is between MZ and DZ pairs, zygosity is not recorded on birth certificates. In order to approximate MZ and DZ twins with the data available, they were classified into like-sexed and unlike-sexed pairs. This approximation is, of course, quite crude inasmuch as only about half of the like-sexed twins will be monozygotic. All of the unlike-sexed twins are dizygotic.

INCIDENCE OF CLEFTS IN TWINS. In an earlier study of clefts in four states (7) a higher incidence of clefts among twins than among singletons was reported. The present data, based on a larger series, do not support that finding. As shown in Table 1, the incidence rates of clefts per 100,000 live births were similar for singletons and twins from like-sexed and unlike-sexed pairs.

SEX RATIOS OF TWINS WITH CLEFTS. If clefts were in some way associated with monozygotic twinning and not with the phenomenon of double occupancy in the uterus, one would expect that any observed differences in the proportion of males and females in twins compared with single births would be attributable to twins from like-sexed pairs. One would not expect any sex differences between single births and twins from unlike-sexed pairs.

Table 2 shows the percentage of males among live born twins and single births for each type of cleft. For isolated cleft lip there was a sug-

TABLE 1. Incidence of clefts in live born individuals by type of cleft and twin status.

<i>type of cleft</i>	<i>affected individuals among</i>					
	<i>like-sexed twins</i>		<i>unlike-sexed twins</i>		<i>single births</i>	
	<i>number</i>	<i>rate¹</i>	<i>number</i>	<i>rate¹</i>	<i>number</i>	<i>rate¹</i>
All clefts	148	110.0	66	101.2	11,122	111.1
Isolated cleft lip	48	35.7	17	26.1	2,957	29.5
Cleft lip and palate	56	41.6	26	39.9	4,757	47.5
Isolated cleft palate	44	32.7	23	35.3	3,408	34.0
Total live births	134,500		65,200		10,009,600	

¹ per 100,000 live births

gestion of fewer males among like-sexed twins compared with unlike-sexed twins and single births. The percentage of males with cleft lip and palate was similar in twins and singletons. For isolated cleft palate, single births and twins from unlike-sexed pairs yielded similar percentages of males, but there was a reversal in the usual sex distribution among twins from like-sexed pairs. This reversal from female to male preponderance was, interestingly enough, also observed in other malformations (8), suggesting perhaps either a higher fetal mortality rate among affected female MZ twins or a positive association between the malformations, male sex, and MZ twinning.

CONCORDANCE FOR CLEFTS. Another item of interest was the degree to which like-sexed pairs were more likely to be concordant for clefts than unlike-sexed pairs. There are two basic methods for computing concordance for a disease, both of which are discussed thoroughly by Allen (3). The most commonly used method is termed the "pairwise" concordance rate, which is simply the proportion of all affected pairs in which both members are affected. When every affected individual in the sample has been independently ascertained, as is the case in the present series, the pairwise concordance rate is defined as the number of concordant pairs divided by the total number of pairs. The quotient is multiplied by 100 for expression as a percentage.

Table 3 shows the pairwise concordance rates of cleft lip with or without cleft palate and of isolated cleft palate. The rates are approximately four times as high for like-sexed pairs as for unlike-sexed pairs. Incidentally, there were three pairs in which one twin had isolated cleft lip and the co-twin a cleft lip and palate, but there were no pairs in which one had isolated cleft palate and the other a cleft involving the lip. This finding adds some support to the hypothesis of independence of these two major types of clefts (5).

The second method of computing concordance rates discussed by Allen is the "proband" method which estimates the probability of a co-twin's

TABLE 2. Number and percentage of males among live born individuals with clefts by type of cleft and twin status.

<i>type of cleft</i>	<i>affected individuals among</i>								
	<i>like-sexed twins</i>			<i>unlike-sexed twins</i>			<i>single births</i>		
	<i>total</i>	<i>male</i>		<i>total</i>	<i>male</i>		<i>total</i>	<i>male</i>	
		<i>no.</i>	<i>%</i>		<i>no.</i>	<i>%</i>		<i>no.</i>	<i>%</i>
All clefts	148	89	60	66	36	55	11,122	6,536	59
Isolated cleft lip	48	25	52	17	10	59	2,957	1,802	61
Cleft lip and palate	56	36	64	26	16	62	4,757	3,134	66
Isolated cleft palate	44	28	64	23	10	43	3,408	1,600	47

TABLE 3. Observed pairwise rates of concordance for clefts by type in like-sexed and unlike-sexed twin pairs.

<i>type of cleft</i>	<i>like-sexed pairs</i>			<i>unlike-sexed pairs</i>		
	<i>total pairs</i>	<i>concordant</i>	<i>concordance rate (%)</i>	<i>total pairs</i>	<i>concordant</i>	<i>concordance rate (%)</i>
All clefts	129	17	13.2	63	2	3.2
Cleft lip with or without cleft palate	93	10	10.8	42	1	2.4
Isolated cleft palate	36	7	19.4	21	1	4.8

Note: Only twin pairs in which both members were born alive are included.

being affected, given a cleft in the index twin. When both twins are ascertained independently, each affected twin must be considered an index case. Thus, the proband concordance rate is defined as the number of affected individuals in concordant pairs divided by the total number of affected individuals (in concordant and discordant pairs). The advantage of this method is that the results can be directly compared to the recurrence rates estimated from family studies. Table 4 shows the proband concordance rates, which are considerably higher than the pairwise concordance rates shown in Table 3. Comparison of Tables 3 and 4 illustrates that the same data can yield different concordance rates when different methods are applied. When calculating concordance rates it is important that the appropriate method be selected and specified.

It is unfortunate that zygosity is not known for the like-sexed twins in this series, inasmuch as the numbers are larger than in previous studies and the sample is relatively unbiased. Nevertheless, it is possible to make estimates of the number of MZ and DZ twins in the series by applying the Weinberg difference method. This method assumes that there is a 1:1

TABLE 4. Observed proband rates of concordance for clefts by type in like-sexed and unlike-sexed twin pairs.

<i>type of cleft</i>	<i>affected individuals in</i>					
	<i>like-sexed pairs</i>			<i>unlike-sexed pairs</i>		
	<i>total</i>	<i>concordant</i>	<i>concordance rate (%)</i>	<i>total</i>	<i>concordant</i>	<i>concordance rate (%)</i>
All clefts	146	34	23.3	65	4	6.2
Cleft lip with or without cleft palate	103	20	19.4	43	2	4.7
Isolated cleft palate	43	14	32.6	22	2	9.1

Note: Only individuals from twin pairs in which both members were born alive are included.

TABLE 5. Estimated pairwise rates of concordance for clefts by type in a hypothetical distribution of MZ and DZ twin pairs based on Weinberg's difference method.

<i>type of cleft</i>	<i>MZ twin pairs</i>			<i>DZ twin pairs</i>		
	<i>total pairs</i>	<i>concordant</i>	<i>concordance rate (%)</i>	<i>total pairs</i>	<i>concordant</i>	<i>concordance rate (%)</i>
All clefts	66	15	22.7	126	4	3.2
Cleft lip with or without cleft palate	51	9	17.6	84	2	2.4
Isolated cleft palate	15	6	40.0	42	2	4.8

Note: Only twin pairs in which both members were born alive are included.

ratio of like-sexed to unlike-sexed pairs among the DZ twins. In addition, it must also be assumed that the rate of concordance for clefting is the same for both like-sexed and unlike-sexed DZ twins.

Table 5 shows the pairwise concordance data to which the Weinberg method for estimating MZ and DZ twins has been applied. As expected, the concordance rates obtained for the estimated MZ pairs are about twice as high as those for all like-sexed pairs shown in Table 3, while the same rates are maintained for the estimated DZ pairs as for unlike-sexed pairs. Because most previously published data on concordance of clefts in twins have been based on the pairwise method, the data in Table 5 can be compared with other studies in which zygosity of individual pairs of twins was presumably established. Among MZ twins with clefts of any type, Metrakos, Metrakos, and Baxter (9) reported concordance of 31%, and Douglas (4) reported concordance of 30%. These data are reasonably comparable to the 22.7% shown in Table 5. The former authors described a higher rate of concordance for clefts involving the lip than for isolated cleft palate, whereas we observed the opposite relation.

Calculation of the proband concordance rate based on the hypothetically distributed MZ and DZ twins in the present series yields data that can be compared directly with published incidence rates of clefts in siblings of affected individuals. Woolf, Woolf, and Broadbent (11) reported that 4.61% of siblings of cleft lip (with or without cleft palate) and 2.55% of siblings of isolated cleft palate probands were also affected. Their data are very similar to Fogh-Andersen's earlier report (5) of 4.91% and 2.51%, respectively. Data from the present study shown in Table 6 yield proband concordance rates for estimated DZ twins with cleft lip with or without cleft palate (4.7%) almost identical to the rates for siblings quoted above. However, the concordance rate for isolated cleft palate among DZ twins (9.1%) is considerably higher than the published rates of affected siblings.

ASSOCIATED ANOMALIES IN TWINS WITH CLEFTS. If an individual has one congenital malformation, the probability of his having additional anoma-

TABLE 6. Estimated proband rates of concordance for clefts by type in a hypothetical distribution of MZ and DZ twin pairs based on Weinberg's difference method.

<i>type of cleft</i>	<i>affected individuals in</i>					
	<i>MZ twin pairs</i>			<i>DZ twin pairs</i>		
	<i>total</i>	<i>concordant</i>	<i>concordance rate (%)</i>	<i>total</i>	<i>concordant</i>	<i>concordance rate (%)</i>
All clefts	81	30	37.0	130	8	6.2
Cleft lip with or without cleft palate	60	18	30.0	86	4	4.7
Isolated cleft palate	21	12	57.1	44	4	9.1

Note: Only individuals from twin pairs in which both members were born alive are included.

lies is much greater than if malformations were associated fortuitously. Studying the association of apparently unrelated anomalies may provide clues to their causes. Twins offer a unique opportunity to study such associations. We were specifically interested in whether twins with clefts had more associated malformations than singletons with clefts and whether there was a higher incidence than expected of other types of malformations among co-twins of individuals with clefts.

One should bear in mind that some malformations, especially heart defects, are not diagnosed at birth, and associations between clefts and such malformations are probably grossly underestimated when ascertainment is limited to birth certificates or other neonatal records.

As shown in Table 7, a somewhat higher proportion of twins with

TABLE 7. Frequency of associated congenital malformations in live born individuals with clefts by type of cleft and twin status.

type of cleft	affected individuals among								
	like-sexed twins			unlike-sexed twins			single births		
	total	with associated malformations		total	with associated malformations		total	with associated malformations	
		no.	%		no.	%		no.	%
All clefts	148	30	20.3	66	13	19.7	11,122	1,725	15.5
Cleft lip with or without cleft palate	104	17	16.3	43	7	16.3	7,714	841	10.9
Isolated cleft palate	44	13	29.5	23	6	26.1	3,408	884	25.9

TABLE 8. Frequency of malformations other than clefts in co-twins of cleft probands by type of cleft and sex type of twin pair.

<i>type of cleft</i>	<i>all co-twins</i>			<i>like-sexed co-twins</i>			<i>unlike-sexed co-twins</i>		
	<i>total</i>	<i>with other malformations</i>		<i>total</i>	<i>with other malformations</i>		<i>total</i>	<i>with other malformations</i>	
		<i>no.</i>	<i>%</i>		<i>no.</i>	<i>%</i>		<i>no.</i>	<i>%</i>
All clefts	211	14	6.6	146	11	7.5	65	3	4.6
Cleft lip with or without cleft palate	146	8	5.5	103	6	5.8	43	2	4.7
Isolated cleft palate	65	6	9.2	43	5	11.6	22	1	4.5

Note: Only twin pairs in which both members were born alive are included.

cleft lip with or without cleft palate had additional anomalies than did their singleton counterparts. With regard to isolated cleft palate, the proportion of individuals having additional malformations was quite similar in all three groups.

The frequency with which co-twins of infants with clefts had one or more congenital anomalies other than a cleft is shown in Table 8. A total of 14 co-twins were thus affected, 11 from like-sexed pairs and 3 from unlike-sexed pairs. Whereas the overall incidence of all malformations reported on birth certificates varies around 1%, in this study 6.6% of co-twins of individuals with clefts had malformations other than clefts. The incidence of other malformations was exceptionally high among like-sexed co-twins of individuals with isolated cleft palate, with 11.6% of these children reported to have a non-cleft malformation.

Among the 14 malformed co-twins shown in Table 8, nine had skeletal anomalies, including three with talipes and two with polydactyly. Thus, 64% of the affected co-twins had skeletal anomalies, whereas, among all live births, skeletal anomalies accounted for 32% of all congenital malformations reported on birth certificates included in the National Cleft Lip and Palate Intelligence Service.

The fact that both unlike-sexed as well as like-sexed co-twins of cleft probands had a high incidence of other types of congenital malformations suggests that environmental factors may play a particularly influential role in those cases.

Summary

Birth certificates of 214 twins with clefts and their co-twins were analyzed to observe differences among twins from like-sexed pairs, those from unlike-sexed pairs, and single births.

The incidence rates of clefts were similar for twins from like- and

unlike-sexed pairs and singletons. For isolated cleft lip there were somewhat fewer males among like-sexed twins than among unlike-sexed twins and singletons. A much higher proportion of males with isolated cleft palate was observed among like-sexed twins than among unlike-sexed twins or single births. The proportion of males with combined cleft lip and palate was similar in twins and singletons.

Concordance rates for clefts, computed by both the "pairwise" and the "proband" methods, were higher for like-sexed than for unlike-sexed twins. The Weinberg difference method was applied to the data to estimate the number of MZ and DZ twins. The pairwise concordance rates for all clefts combined were similar to previously published rates. The proband concordance rate among estimated DZ twins with cleft lip with or without cleft palate was similar to published incidence rates among siblings of affected individuals, whereas the rate for isolated cleft palate was higher than published incidence rates of siblings.

Twins with clefts involving the lip were reported to have additional anomalies more often than singletons, but no difference in the frequency of associated malformations was noted between twins and singletons with isolated cleft palate. The frequency of malformations other than clefts was much higher in co-twins of cleft probands than in the general population of newborns.

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