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The Association of Twinning and Neural Tube Defects:
Studies in Los Angeles, California, and Norway

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Accurate, unbiased malformation rates in twins must be obtained unselectively from population-based studies that include livebirths and stillbirths after a thorough ascertainment of cases. This type of study was conducted in Los Angeles County, California, where 28 twins with a neural tube defect (NTD) were identified. The prevalence in twins (1.6/1,000) was significantly higher than in singletons (1.1/1,000). The study then was expanded to include population-based data from the Medical Birth Registry of Norway which has a comparable overall NTD prevalence (1.0/1,000) and twinning rate (2%). The combined material shows a higher prevalence of anencephaly and encephalocele but not of spina bifida in twins compared to singletons. The male/female ratios in total twin and singleton cases were comparable (0.8), but varied by specific defect. Like-sex twin females appeared at highest risk for NTD as well as for fetal death.

This study supports theories which associate NTDs with monozygotic twins, either through developmental disruptions that cause susceptibility to environmental agents or through a common etiology. Furthermore, it suggests that twins and singletons differ in their response to etiologic factors for the development of NTDs and that the development of each type of NTD may be related to different factors.

Key words: Neural tube defects, Twinning, Anencephaly, Spina bifida, Encephalocele, Case ascertainment, Malformations in twins

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INTRODUCTION

The epidemiology of the neural tube defects (NTD), anencephaly, spina bifida and encephalocele is complex. A variety of etiologic hypotheses have been offered, including genetic [4], environmental [19], and multifactorial [21] insults. The proportion of genetic and environmental factors involved in NTDs may vary by different populations. Low rates of NTDs in some geographic locations may represent baseline levels primarily determined by genetic factors, whereas higher rates in other areas possibly may be attributed to environmental factors in addition [15].

Twin studies traditionally have been used to help distinguish hereditary from environmental components in the causation of a given disease. The value of twin studies for congenital malformation research is based on the assumption that all co-twins share similar prenatal environments. Monozygotic (MZ) twins also share identical genotypes, whereas dizygotic (DZ) twins are no more similar genetically than other siblings. In addition to clarifying the contributions of genetic and environmental factors, the study of twins and NTDs is of particular interest because the twinning process itself may be etiologically associated with the development of congenital malformations [12]. One of the difficulties with twin studies in the past has been the need to obtain unselected cases in numbers great enough for studying relatively rare malformations.

A study designed to overcome the problem of selection bias by identifying all twin births (live and dead) that occured in a defined population was initially conducted in Los Angeles County, California, from 1966 through 1972 [26]. Twenty-eight NTD cases among twins were identified; this number yielded a significantly higher NTD prevalence among twins (1.6/1,000) than among singletons (1.1/1,000). Since this increase was not in agreement with the general observations in the literature, [3,11,15,17,28], it warranted confirmation with a larger number of cases. The study thus was expanded to include population-based data available from mandatory birth registration in Norway where a low overall NTD rate (1.0/1,000) also had been observed.

MATERIALS AND METHODS

Population figures for Los Angeles were obtained from the Bureau of Records and Statistics, and cases were independently ascertained from vital records as well as hospital records [26]. Twin NTD cases were identified by information in the case records and then were matched to their unaffected co-twins. "Fetal deaths" or stillbirths were reported among fetuses of 20 or more weeks' gestation.

Data about the Norwegian population and the NTD cases were derived from the Medical Birth Registry of Norway [1] for the period 1967 to 1979. In Norway, reporting of all fetuses of 16 weeks' gestation or more is mandatory. The required data includes demographics about the parents and the fetus, as well as the mother's health before and during pregnancy, circumstances surrounding the delivery, and the condition of the newborn.

All NTD cases were abstracted from the data base and twins then were identified from information coded on the case records. The total twin population was separated from the general population and a working computer file was created to aid further analysis.

In this study, the term "prevalence rate" is used to indicate malformation prevalence-at-birth; true malformation incidence rates are not known since affected fetuses may be aborted spontaneously early in gestation [23]. All rates were calculated for individual births rather than pairs. Cases of anencephaly with spina bifida or encephalocele were classified as anencephaly. Cases of spina bifida with encephalocele were classified as

encephalocele in order to study encephaloceles as one group. Information on zygosity was not available from either data source. However, because like-sex (LS) pairs include all monozygous (MZ) pairs as well as approximately half the dizygous (DZ) pairs, differences attributed to LS pairs may be even greater among MZ twin pairs.

The data from these two sources are presented as combined material, but results from the separate analysis also will be mentioned. Since the NTD rate and the twinning rate are very similar in these two populations of nearly equal size, pooling not only is justifiable but desirable. If indeed low NTD prevalence areas primarily yield baseline rates, environmental differences between the two areas should not be critical. Although the Norwegian births are reported over a longer span of time, NTD rates have not changed significantly during this period in Norway [27]. Another factor of interest is that the Norwegian population is much less racially varied than the Los Angeles population.

Chi-square and tests of proportions were used to examine the importance of noted differences between twins and singletons.

RESULTS

In Norway, 790,995 births occurred from 1967 to 1979, compared to 865,351 births among Los Angeles County residents from 1966 to 1972. Of the Los Angeles births 16,880 (1.95%) were members of matched twin pairs, whereas 15,320 (1.94%) of Norwegian births were twins. Combined, these two areas provided a total of 32,200 twin individuals for study.

There were 28 twin NTD cases among 962 NTD cases in Los Angeles and 23 twin cases among 795 NTD cases in Norway. Each area had one triplet NTD case included in the totals. In Los Angeles, members of twin pairs constituted 3.4% of anencephaly cases, 1.4% of spina bifida cases and 9.7% of encephalocele cases compared to 3.2% of anencephaly cases, 3.1% of spina bifida cases and none of the encephalocele cases in Norway. In the combined material, a total of 2.9% twins among NTD cases was observed; this figure is greater than the twinning rate for the respective populations. With a twinning rate of approximately 2%, 35 twins would be expected among 1,757 NTD cases, whereas 51 were actually observed.

The prevalence of NTDs is compared for twins and singletons by specific defect in Table 1. The Los Angeles figures are available in reference 26 and the Norwegian figures

TABLE 1. NTD Cases Among Twin and Single Births. Prevalence per 1,000 Births in Norway and Los Angeles Combined

	Twin Births					Single Births		Relative Risk
	Like-sex		Unlike-sex					
	Cases	Rate[b]	Cases	Rate	Total twin[a] rate	Cases	Rate	Twin versus singleton
Anencephaly	20	0.89	5	0.52	0.78	777	0.48	1.6 ($P < .025$)
Spina bifida	14	0.62	4	0.42	0.56	816	0.50	1.1 (NS)
Encephalocele	7	0.31	0	0.00	0.22	112	0.07	3.1 ($P < .01$)
Total NTDs	41	1.82	9	0.94	1.58	1,705	1.05	1.5 ($P < .001$)
Total births	22,548		9,618		32,200	1,623,950		

[a]Includes unknown pair status.
[b]Rate = prevalence per 1,000 births.

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are available partially in reference 27 and by subtraction if desired. The prevalence of NTDs is similar in the two populations; the overall NTD rate is 1.05/1,000 births. For each specific defect, the prevalence in like-sex twins is greater than that in unlike-sex (US) twins. The pooled data show a significantly higher rate in twins than singletons for anencephaly ($P < .025$), encephalocele ($P < .01$) and total NTDs ($P < .001$), but the rates for spina bifida are not significantly different.

The differences between the two populations are as follows: In Norway spina bifida is increased in twins compared to singletons, whereas encephalocele is decreased; in Los Angeles spina bifida is decreased in twins and encephalocele is greatly increased. Statistical comparison of the different proportions of each defect between the two populations reveals that the spina bifida findings may be due to chance or sampling ($P = .1$). The differences in encephalocele do not, however, appear to be due to chance ($P < .01$), even when "encephalocele excluding spina bifida" rates are compared ($P < .05$).

The distribution of cases by sex and defect is displayed in Tables 2, 3. As expected [5], more females than males were affected. However, the differences between sexes are statistically significant among singletons only for anencephaly and total NTD cases and among twins only for encephalocele cases (Table 3). The male/female ratio is not significantly different between twins (.85) and singletons (.79) for overall NTDs (Table 2).

TABLE 2. Male/Female Sex Ratio in Twin Compared to Singleton NTD Cases and Births in Norway and Los Angeles

	Twins		Singletons	
	No. of Males:females	M/F Ratio	No. of Males:females	M/F Ratio
Anencephaly	10:15	0.67	300:474	0.63
Spina bifida	12:06	2.00	389:418	0.93
Encephalocele	1:06	0.17	59:53	1.11
Total NTDs	23:27	0.85	748:945	0.79
Population	16,089:16,091	1.00	833,208:790,614	1.01

TABLE 3. Prevalence of Neural Tube Defects by Sex in Twins Versus Singletons in Norway and Los Angeles

	Twin births			Single births		
	Prevalence/1,000		RR[a] of NTD	Prevalence/1,000		RR[a] of NTD
	Male	Female	Female vs male	Male	Female	Female vs male
Anencephaly	0.62	0.93	1.5	.36	.60	1.7 ($P < .001$)
Spina bifida	0.75	0.37	0.5	.47	.53	1.1 (NS)
Encephalocele	0.06	0.37	6.2 ($P = .05$)	.07	.07	1.0 (NS)
Total NTDs	1.43	1.68	1.2	.90	1.20	1.3 ($P < .001$)

[a]RR = relative risk.

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Comparing twin to singleton sex ratios by defect it was found that among anencephaly cases the ratios are comparable, but among spina bifida cases the twin M/F ratio is greater than the singleton ratio. Among encephalocele cases, on the other hand, the twin M/F ratio is much lower than the singleton M/F ratio. The sex variations among twins may be due to the small number of cases since the singleton sex ratios from these populations are within the expected range for each type of NTD.

As shown by the calculations in Table 4, female NTD cases are significantly more likely to be stillborn than male cases, particularly among like-sex twins. In contrast, in the twin and the singleton populations, males have an equal or greater risk of being stillborn than females. The pooled data shows approximately equal rates of stillborns in twin and singleton NTD cases. When examined by sex, however, female twins include a greater percent of fetal deaths than female singletons, whereas male twins have a lower percent of stillbirths than male singletons.

In each population there is one twin pair concordant for NTDs yielding a pairwise concordance rate of 4.1% (2/49 pairs). Both pairs are like-sex, one male and one female, so the concordance rate among like-sex pairs is 5.3% (2/38 pairs). If both pairs are monozygotic, the estimated MZ concordance would be 6.9% (2/29 pairs, obtained by subtracting the number of US pairs from the LS pairs). Also, both concordant pairs have spina bifida, although each member of the female-female pair from Los Angeles has both spina bifida and encephalocele.

DISCUSSION

Studies of congenital malformation rates in twins have been hampered by the small number of available cases, especially if only one defect is being looked for. In an attempt to

TABLE 4. Percent of Stillbirths Among Singletons, Like-Sex and Unlike-Sex Twins by Sex, in Norway and Los Angeles

	Male		Female			
	Total number	Stillborn (%)	Total number	Stillborn (%)	Total stillbirths (%)[a]	Relative risk of stillbirths in female vs male
Twin NTDs	23	13.0	27	51.0	35.3	4.00[b]
Like-Sex	19	5.3	22	54.6	31.7	10.30[c]
Unlike-Sex	4	25.0	5	40.0	33.3	1.60
Twin population[a]	16,089	4.2	16,091	4.2	4.5	1.00
Like-Sex	11,288	5.5	11,284	4.8	5.2	0.87[d]
Unlike-Sex	4,797	2.8	4,797	2.5	2.6	0.89
Singleton NTDs	748	29.0	945	39.1	35.0	1.30[c]
Singleton population	833,457	1.3	790,324	1.1	1.2	0.85[c]

[a]Includes unknown sex or pair type.
[b]$P < .01$.
[c]$P < .001$.
[d]$P < .025$.
Others not significant.

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derive accurate prevalence rates of neural tube defects in twins, this study used population-based data with cases and twin status ascertained independently. Cases were included from fairly early in gestation rather than just live births, which is important because there appear to be differential rates of fetal death by sex and plurality. Although pooling two data sources may obscure some subgroup differences, this methodology alternatively may provide a more representative picture not available from smaller case series.

Findings

Our most important finding was higher prevalence of NTDs observed in twins compared to singletons. Of particular interest was the increase in LS pairs compared to US pairs, suggesting a higher prevalence of NTDs in MZ versus DZ twins.

In reviewing the literature [2,3,7–11,16], the excess of twins among NTD cases is supported by studies that include fetal deaths and are from lower NTD prevalence areas but this finding is not as consistent in higher-prevalence areas. By defect type, the excess of twins appears most frequently among anencephaly cases, the severest of the neural tube defects. For spina bifida and encephalocele which often are grouped together, the percent of twins varies considerably between different studies, so that no clear pattern emerges. Nonetheless, spina bifida rates in twins appear to be similar to those in singletons, whereas encephalocele may be increased in twins [16,26]. Categorizing twin encephalocele cases from Los Angeles by race reveals that three of seven cases, or three of four cases of encephalocele excluding spina bifida, are listed as Caucasian-Spanish (Hispanic). Although data on NTD rates in Hispanics are sparse, two investigations have found higher rates of total NTDs in Hispanics living in Los Angeles [25] and in New York [6]. Since Hispanics are rarely present in Norway, further examination of encephalocele by race seems warranted.

As expected, more females than males with NTD were found. A low male/female ratio is consistent among twin and singleton anencephalics, and the ratio is about one for singleton spina bifida and encephalocele cases. Like the prevalence data, however, the sex ratios of twin spina bifida and encephalocele cases exhibit some variation among the two populations, perhaps due to smaller numbers.

Like-sex twin females appear to be at increased risk for a NTD, particularly anencephaly; among NTD cases, they are also at highest risk for fetal death. Even among purely anencephalic twins, the percentage of fetal deaths among females is still greater than in males, with a relative risk of 4.0 ($P < .01$). Twin female cases also have a higher percentage of stillbirths than singleton female cases, suggesting that female twins are more "susceptible" or represent a high-risk group. On the other hand, instead of being more "susceptible," females may just survive to be counted, whereas males may be more likely to be aborted early in gestation. However, the low rate of stillbirths in male anencephalics that do reach registerable gestational age and the increase of males over females in the Norwegian twin data would not seen to fit this suggestion.

The finding of concordance among only LS pairs is of interest as it relates to MZ twins, and tends to indicate a hereditary association. However, the large number of nonconcordant affected LS twins is more suggestive of an association of MZ twinning itself with the development of a NTD.

Conclusions and Comments on Etiology

An association of the MZ twinning process and congenital malformations has received attention recently. Myrianthopoulos [18] proposed that the MZ twinning process disrupts

the developmental clock, creating disadvantages in the two embryos that render them more susceptible to the action of subtle environmental agents. Schinzel et al [20] suggested that there is a common etiology for the MZ twinning process and early malformations such that an early insult causing duplication can lead to additional morphologic problems. The findings of this study regarding female, like-sex twins lends support to James's suggestion [13] that there is a common factor, namely, developmental delay, between NTDs, females, and MZ twins.

Although DZ rather than MZ twinning generally is considered to have a familial basis, recently there has been a report [24] of familial MZ twinning. As suggested in the Los Angeles study [26], if twins and MZ twins in particular are more susceptible to environmental insults (either genetically or through embryologic disruptions), then lower exposures of such agents might be sufficient to cause neural tube defects in twins but not in singletons. Thus an excess of twin cases might be expected in low NTD prevalence areas, where adverse environmental factors may be present at low levels [15,22]. In areas with higher NTD prevalence and a possibly greater etiologic role for environmental factors, more singletons would become affected and obscure the association with twinning. The variations by type of neural tube defect between twins and singletons support the suggestion [26] of an etiologic mechanism related to twins or twinning that is manifested differently for the specific defects. James [14] has recently developed an hypothesis with a similar conclusion.

To further investigate the association of MZ twinning with congenital malformations, we plan to study other types of birth defects in twins from the population-based Norwegian data. Elevated rates in LS pairs that are not concordant would further implicate an association with the MZ twinning process, whereas a large number of concordant pairs would be more suggestive of a genetic component.

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