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A Familial Association between Twinning and Upper–Neural Tube Defects

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Summary

An increased twinning rate has been observed in the near relatives (sibs, parents, and aunts and uncles) of probands with neural tube defects (NTDs) occurring at the level of the 11th thoracic vertebra and above (upper NTDs). The twin rate was more than double that of the near relatives of probands with lower NTDs and of those of probands with Mendelian disorders (the controls). The excess twinning was same sex and can therefore consist of either MZ or same-sex DZ twins. Furthermore, upper-NTD families with twins had a higher NTD-sibling occurrence rate than did families without twins. These findings, if corroborated, may imply an etiology common to twinning and NTDs and can perhaps be applied in counseling NTD families.

Introduction

Neural tube defects (NTDs) and twinning both arise early in human embryonic development. That one may be causally related to the other has been suggested by the fact that they occur together more often than expected by chance. For example, an increased prevalence of anencephaly (AN) has been observed in same-sex twins (James 1976; Layde et al. 1980). In an exhaustive review by Little and Elwood (1992), these and other studies suggesting such a link were viewed with skepticism, partly because of the statistical limitations of the data. Nevertheless, some hypotheses have been proposed to account for such an association, most notably that NTDs arise as a consequence of zygotic fission and/or twin embryogenesis or as a result of interactions between gestating co-twins (summarized by Little and Elwood 1992).

Other reported associations include increased rates of NTD in the sibs of DZ twins (Fraser and Hanson 1984; Windham and Bjerkedal 1984), an excess of DZ twinning in the mothers of probands with spina bifida (SB) (LeMarec et al. 1978), and an excess of same-sex twins among the mothers of children with midline neural defects (Corey et al. 1980). Also, there appears to be an increase in nonright-handedness in the parents of twins, both MZ and DZ (Boklage 1981), and in the parents of probands with NTD (Fraser 1983).

Even more indirect evidence of an association exists, albeit weak in many instances. For example, declining secular trends for birth frequency of anencephalics and DZ twins have been observed in the United States and Canada (Elwood 1974) and in Australia (Field and Kerr 1974). Several teratogens, such as vitamin A, dimethyl sulfoxide, and urethan (Ferm 1969), as well as vincristine sulfate (Kaufman and O'Shea 1978) in rodents; certain conditions of altered temperature and oxygen concentration in fish (Stockard 1921; Ingalls et al. 1969); and experimentally delayed fertilization in amphibians (Witschi 1952) have all been observed to increase the MZ twinning rate, in addition to causing NTDs (Shepard 1992). Finally, increases in NTD rate (Lancaster 1987; Cornel et al. 1990; Milunsky et al. 1990) and in MZ twinning rate (Edwards et al. 1986; Derom et al. 1987) have been observed in humans after in vitro fertilization (implying delayed fertilization), in addition to the expected rise in DZ twin rate. In this study, we investigated both whether a familial association exists between twinning and NTDs and whether twinning is a familial risk factor for NTD.

Subjects and Methods

Our sample consisted of 227 nonsyndromic NTD probands, referred to the genetics unit of The Montreal Children's Hospital over the period 1955–89, and their families (Garabedian and Fraser 1993). Information was recorded routinely on first-, second-, and third-degree relatives, preliminarily to genetic counseling. The crude twinning rate (i.e., unadjusted for maternal age and parity) in the sibs, parents, and aunts and uncles of the probands was measured from the pedigrees by counting the total number of twin pairs and dividing by total deliveries, including stillbirths. It did not seem appropriate to use the rate of twinning at birth observed in the general population as the expected rate of twinning in the near relatives of the NTD probands. Postnatal mortality and reduced fertility would reduce the prevalence of twinning in parents, and there could be underreporting of twinning by those providing the family histories. We therefore chose

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Table 1

Twinning Rates in Near Relatives of Probands with NTDs

	% Twinning (No. of Cases)			
	All NTD	Upper NTD	Lower NTD	Control
Sibs	1.0 (405)	2.0 (198)	0 (207)	.7 (554)
Parents	1.1 (450)	1.7 (234)	.5 (216)	1.1 (568)
Aunts and uncles	1.4 (1,726)	2.3* (838)	.7 (888)	1.0 (2,194)
Combined	1.3 (2,581)	2.1** (1,270)	.5 (1,311)	1.0 (3,316)
"Confidence interval"	.9–1.8	1.4–3.1	.2–1.1	.7–1.4

* $P < .003$.
** $P < .004$.

to use, as a comparison group, the families of probands with various Mendelian disorders not associated with twinning, such as cystic fibrosis, Duchenne muscular dystrophy, and hemophilia, seen during the same time span and by the same interviewers, who had no preconceived views of a relationship between NTDs and twinning. Co-twins of parents and co-twins of NTD probands were excluded from the aunts-and-uncles group and the sib group, respectively.

Because of the suggestion by Toriello and Higgins (1985) that SB can be subdivided into a neurulation type (upper SB and encephalocele), if the lesion is at T11 or above, and into a canalization type (lower SB), if it occurs at T12 or below, and including anencephaly in the upper group, following classification III of Hall et al. (1988), we divided our sample accordingly and recalculated twinning rates. Families were also grouped into those with or without twins (in the first-, second-, and third-degree relatives of the proband), and the NTD-sib occurrence rate was calculated by counting all affected sibs of the NTD probands and dividing by the total number of sibs, including stillbirths. This was also done after a subgrouping of the families, according to whether the proband had an upper or lower NTD.

Results

As shown in column 1 of table 1, the twinning rate was not significantly higher (*a*) in any of the individual groups of the relatives of NTD probands than in the relatives of the control probands (whose rates approximated the expected rate of 1.0% for Canada for the years in question [Elwood 1973]) or (*b*) when the data for all relatives were combined (1.3% vs. 1.0%). There were, however, significantly more twins ($P<.004$) in the combined upper-NTD group than in the combined lower-NTD and the combined control groups (2.1% vs. 0.5% and 1.0%, respectively; table 1). The excess was also significant in the aunts-and-uncles group alone (2.3 vs. 1.0%; $P<.003$), with no difference between the paternal and maternal sides. The twin excess was not due to a small number of families with a large number of twins in the upper-NTD group. Fewer twins were found in the combined lower-NTD group than in the control group (0.5% vs. 1.0%), but this difference was not significant.

Since twins were ascertained retrospectively from hospital charts, zygosity tests had not been done on most of them. There was, however, a same-sex excess (data not shown) in the combined upper-NTD group: 76% versus the expected rate of 67.5%, calculated by assuming that 50% of DZ twins are of the same sex (Bulmer 1970) and that ~35% of all twins are MZ (Little and Elwood 1992). This excess could consist either of MZ pairs or of same-sex DZ pairs, an excess that, according to James (1971), would be expected.

NTD-sibling occurrence rates were not significantly higher in families with twins (table 2, line 1). However, when the data were subdivided according to whether the proband had an upper or lower NTD, an increased NTD rate was observed in the sibships of upper-NTD probands (9.1% vs. 3.1%; $P<.03$).

Discussion

It was recently demonstrated that the cutoff point between neurulation and canalization in humans is probably

Table 2

NTD-Sibling Occurrence Rate in Families With and Without Twins

	% Occurrence (No. of Sibs)	
Proband with	Families with Twins	Families without Twins
NTD	7.7 (130)	4.6 (327)
Upper NTD	9.1* (66)	3.1 (163)
Lower NTD	6.3 (64)	6.1 (164)

* $P < .03$.

as low as the level of the second sacral vertebra (Müller and O'Rahilly 1987), indicating that most NTDs are of the "upper" type. Although the upper/lower distinction proved to be arbitrary with respect to developmental mechanism, it served to reveal, in our sample, a previously unreported excess of same-sex twins in the relatives of probands with defects of the "upper" but not of the "lower" neural tube. No significant excess of twins was observed when NTDs were divided into AN and SB subgroups, in contrast to the findings of Le Marec et al. (1978) and, subsequently, of Journel et al. (1985), who reported an opposite-sex twin excess in the mothers (but not in the fathers) of SB cases. Corey et al. (1980) also found an excess of twins in mothers of children with midline neural defects, but of same sex twins. Maternal/paternal differences in twin rate were not observed in our study. It would be interesting if the data presented from the above-mentioned groups showed differences when subdivided (*a*) into "upper" and "lower" NTDs or (*b*) according to the closure sites at various levels suggested by Van Allen et al. (1993). The latter classification has not been validated, however, nor has a difference in either sex ratio or recurrence risks for the postulated different closure sites been demonstrated. Showing differences, in sex ratios and recurrence rates, between the five postulated levels, much less in twinning rates in relatives, would present serious difficulties, both in accurate establishment of level of closure retrospectively and in getting enough cases to provide adequate numbers for each level. It is difficult enough to obtain sufficient data to make robust comparisons even when only two or three levels are used (Park et al. 1992; Garabedian and Fraser 1993).

If our findings are confirmed in other populations, they may provide some clues to the etiology of both NTDs and twinning. Until then, one can speculate that some familial factor predisposes to upper NTDs and twinning, either MZ or same-sex DZ. The factor would have to act very early in embryonic life, perhaps in the form of a developmental delay, as suggested by James (1975). In either case, upper NTDs would be involved because the upper neural tube forms earlier than does the lower and would therefore be more susceptible to the effects of an early delay than would the lower neural tube. This would also explain the observed female excess and increased rate of additional malformations in upper-NTD probands, an explanation proposed recently by Garabedian and Fraser (1993).

If the association turns out to be with MZ twins, possible reasons for a delay may be delayed ovulation (Bomsel-Helmreich and Papiernik-Berkhauer 1976), delayed fertilization (Stockard 1921; Witschi 1952), or delayed implantation (James 1975), all of which could be genetically influenced. Delayed ovulation or delayed fertilization would result in overripe ova, which are believed to lack cohesion, and this could cause the splitting of the zygote. Similarly, decreased cohesion, or "stickiness," of neurulating cells could produce NTDs. Delayed implantation may also result from reduced stickiness of the fertilized egg.

Of course the above hypothesis would require that MZ twinning itself be heritable, at least in some families. Although this is generally not observed, there are a few reports of familial MZ twinning (Harvey et al. 1977; Segreti et al. 1978; Shapiro et al. 1978; Parisi et al. 1983). MZ twins may be very common early in embryogenesis, but they may only rarely develop to a detectable stage and may even more rarely reach term, which would lead to underestimation of familial MZ twinning.

If, on the other hand, the familial association of upper NTDs is with same-sex DZ twins, it is relevant that delayed fertilization has also been implicated in DZ twinning (Harlap et al. 1985). The heritable factors in this case might be hormones that play a role in controlling the timing of ovulation or fertilization.

Since the historical AN/SB grouping may have obscured or minimized an association between NTDs and twinning in previous reports, more studies are required to see whether our finding represents a true association and whether the association is between upper NTDs and MZ twinning, between upper NTDs and DZ twinning, or both, as suggested originally by Boklage (1987). A familial association between twinning and upper NTDs would have a practical application in counseling if upper-NTD probands with twin relatives have a higher risk of subsequent NTD than do those without them.

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