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Etiologic Heterogeneity of Neural Tube Defects. II. Clues from Family Studies

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SUMMARY

We previously reported that among neural tube defects (NTDs) with no known causes the ones that occur alone (singles) have different epidemiologic characteristics from those that occur in combination with other defects (multiples), suggesting an underlying causal heterogeneity. In this study, we compared family histories of 223 single NTD cases and 66 multiple cases ascertained through the Metropolitan Atlanta Congenital Defects Program (MACDP) between 1970 and 1979. Compared with siblings of multiples, siblings of singles had a higher precurrence rate for NTDs (2.0% vs. 0.0%) and for birth defects in general (10.9% vs. 3.0%). Furthermore, siblings of singles that were born within 2 years before the birth of the index case had a higher precurrence rate for NTDs (8.0% vs. 1.1%) and for major birth defects (20.0% vs. 2.9%) than had those born earlier. These results further suggest that NTDs are etiologically heterogeneous, depending on the presence of associated defects, and point to important environmental influences in the increased risk for birth defects among siblings of singles. Larger studies are needed to confirm these data and show that single and multiple NTDs have different recurrence rates, not only for NTDs but also for other birth defects.

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INTRODUCTION

In [1], using data from two birth defects surveillance systems in the United States, we divided neural tube defects (NTDs) that have no recognized causes into two groups, singles and multiples, according to the presence of associated major defects. Only singles, which accounted for the majority of the cases, were found to have the well-known epidemiologic characteristics of NTDs [2]: marked predominance of females and whites, geographic variation with an east-to-west gradient, and decreasing rates over time. In contrast, multiples had no excess of females and occurred less predominantly in whites; moreover, their rates showed no geographic variation and little or no downward trends over time. Since these epidemiologic differences may indicate an underlying etiologic heterogeneity, we conducted our present family study by using maternal interview data from the Metropolitan Atlanta Congenital Defects Program (MACDP). The two groups were shown to have different sibling precurrence rates for birth defects in general.

MATERIALS AND METHODS

The MACDP is a surveillance system that monitors the occurrence of birth defects in the central five-county metropolitan Atlanta area. This system has been operating since 1968. Cases include all major birth defects diagnosed within the first year of life. Multiple methods of ascertainment are used, including review of maternal and child hospital records, vital records, and cytogenetic results. Because of adequate follow-up, case ascertainment is considered essentially complete. Detailed information on MACDP is available [3].

Since 1970, we conducted interviews with mothers of children with a selected group of birth defects, including NTDs, searching for clues to etiology. Information was collected on prenatal, maternal, and paternal events, exposures, and illnesses. Inquiries also included maternal reproductive history, parental consanguinity, and the occurrence of birth defects, cancers, and common illnesses among various family members. The family data were used by one of us (J. D. E.) in a study of ethnic differences in the occurrence of birth defects [4].

For our study, we reviewed family histories of NTD cases ascertained from 1970 to 1979. We included cases of anencephaly, spina bifida (meningocele, meningomyelocele), and encephalocele. Cases were classified into two groups using our previous criteria [1]: (1) singles, if they had no associated malformations or only defects that were considered minor or secondary to the NTD, and (2) multiples, if they had additional major malformations. As in [1], we excluded cases that had recognized causes (single gene disorders, chromosomal anomalies, intrauterine infections, and known teratogens).

To assess the reliability of family histories obtained from the mother, we attempted independently to ascertain siblings of NTD index cases with major birth defects, using records of the MACDP surveillance system. This could be done only for siblings with major anomalies born after 1968 to mothers who are residents of the metropolitan Atlanta area. Ascertainment was achieved by using the mother's and father's names to link birth defects cases in the system.

RESULTS

Of a total of 364 NTD cases ascertained between 1970 and 1979 that were scheduled for maternal interviews, four had recognized causes (three Meckel's syndrome, one chromosomal anomaly) and thus were excluded from further analysis. Completed interviews were available on 289 cases (223 singles and 66 multiples). Reasons for not interviewing the mother are listed in table 1. Cases for which

TABLE 1

NEURAL TUBE DEFECT CASES, METROPOLITAN ATLANTA, 1970–1979, BY MATERNAL INTERVIEW RESULT

	No. cases	%
Interview refused by physician	19	5.2
Interview refused by family	31	8.5
Not located	14	3.8
Other	11	3.0
Interviewed	289	79.4
Total	364	100.0

no maternal interview was obtained were similar in their sex and race composition and in their proportion of singles and multiples to the ones with completed interviews.

Maternal reproductive history is summarized in table 2. Mothers of singles and multiples had 245 and 82 previous pregnancies, respectively. Of these, 17% in both groups ended in spontaneous abortions and 1% in stillborn infants. Parental consanguinity was reported in one case (single).

The precurrence rates for birth defects among siblings of NTD index cases are shown in table 3. Observed precurrences were compared with the numbers expected based on MACDP population rates. Singles had a full sibling precurrence rate for NTDs of 2.0% (approximately 11 times the expected rate, $P < .0001$), but multiples had no sibling precurrence for NTDs. With these small numbers, however, the difference in sibling precurrence rates for NTDs between singles and multiples did not reach statistical significance. Furthermore, compared with siblings of multiples, siblings of singles tended to have higher precurrence rates for other

TABLE 2

REPRODUCTIVE HISTORY OF MOTHERS OF NTD INDEX CASES, BY CATEGORY OF MALFORMATION

	No. index cases	No. previous pregnancies	% Miscarriages*	% Stillbirths*
Anencephaly:				
Singles	82	92	18.5 (17)†	0.0 (0)
Multiples	20	14	21.4 (3)	0.0 (0)
Spina bifida:				
Singles	128	141	16.3 (23)	2.1 (3)
Multiples	36	51	13.7 (7)	0.0 (0)
Encephalocele:				
Singles	13	12	8.3 (1)	0.0 (0)
Multiples	10	17	23.5 (4)	0.0 (0)
All NTDS:				
Singles	223	245	16.7 (41)	1.2 (3)
Multiples	66	82	17.1 (14)	0.0 (0)

* % previous pregnancies.
† No. cases in parentheses.

TABLE 3

PRECURRENCE RATES OF BIRTH DEFECTS AMONG SIBLINGS OF NTD INDEX CASES, BY CATEGORY OF MALFORMATION: COMPARISON WITH MACDP POPULATION RATES

Defect	Singles	Multiples	MACDP
NTDs	2.0%* (4)†	0.0% (0)	0.18%*
Other major defects	5.0% (10)‡	1.5% (1)	3.5%
Minor defects	4.5% (9)	1.5% (1)	...
All birth defects	10.9%§ (22)	3.0%§ (2)	...

* Comparison of NTD rates between singles and MACDP, $P < .0001$ (cumulative Poisson distribution).

† No. cases in parentheses.

‡ Includes one case with spina bifida and ventricular septal defect.

§ Comparison of rates of all defects between singles and multiples. $P = .0418$ (Fisher's one-tailed test).

major defects (5.0% vs. 1.5%) and for minor anomalies (4.0% vs. 1.5%). The overall precurrence rate for birth defects was 10.9% for siblings of singles and 3.0% for siblings of multiples ($P = .0418$). A variety of birth defects accounted for the excess cases seen among siblings of singles (APPENDIX).

Of the 15 siblings with major birth defects ascertained from maternal interviews, only six were born after 1968 to residents of metropolitan Atlanta and thus could have been independently ascertained through MACDP. All of these were identified in the surveillance system records. The types of defects were mentioned by the mothers with a good degree of accuracy. No further defect cases could be independently ascertained among siblings from the surveillance system review.

Of the birth defect cases among siblings, two occurred in the same family (both were umbilical hernias). There was only one sibling that had two defects (spina bifida with ventricular septal defect).

The precurrence rates for birth defects among siblings of singles were examined for evidence of temporal trends (table 4). We found that siblings born within the 2-year period preceding the birth of the index case had higher precurrence rates for NTDs (8.0% vs. 1.1%, $P = .076$) and for other major birth defects (20.0% vs.

TABLE 4

PRECURRENCE OF BIRTH DEFECTS AMONG SIBLINGS OF SINGLES BY TIME INTERVAL BETWEEN THE BIRTH OF THE SIBLING AND THE INDEX CASE

BIRTH DEFECTS	TIME INTERVAL		TOTAL
	≤ 2 yrs	> 2 yrs	
NTDs	8.0%* (2)†	1.1%* (2)	2.0% (4)
Other major defects	20.0%‡ (5)	2.8%‡ (5)	5.0% (10)
Minor defects	4.0% (1)	4.5% (8)	4.5% (9)
All defects	28.0% (7)	8.5% (15)	10.9% (22)

* Fisher's one-tailed test, $P = .076$.

† No. cases in parentheses.

‡ Fisher's one-tailed test, $P = .003$.

2.9%, $P = .003$) than did those born earlier. There was no similar temporal variation in the rates of minor defects and maternal miscarriages.

The precurrence rates for birth defects among parental siblings are shown in table 5. Among both singles and multiples, maternal siblings tended to have higher precurrence rates for NTDs and other major defects than did paternal siblings. Among singles, maternal siblings had higher NTD rates than expected (based on MACDP rates). However, singles and multiples did not have different precurrence rates for NTDs and other major defects among both maternal and paternal siblings.

Other family variables compared between singles and multiples included sex ratios, twinning rates, and the occurrence of cancers and common diseases among siblings of the index case and the parental siblings. None of these was found to be different.

DISCUSSION

Family studies of NTDs have shown differing estimates of sibling risks for NTDs, ranging from less than 1% to 10% [5]. Generally, lower rates are reported in North America [6, 7] than in the United Kingdom [8, 9]. However, most studies have grouped NTDs together in arriving at such estimates. For a proportion of cases with genetic causes, these figures are not applicable. For example, the sibling risk in cases of Meckel syndrome is 25%, a much higher risk than the range mentioned above. Holmes, in a series of 106 NTD cases [10], found an overall sibling precurrence rate of 5.2%. However, after he excluded 12% of his cases that had recognized causes (half of these were cases of Meckel syndrome), the rates became 1.7% for NTD cases that occur isolated and 0.0% for cases that occur with other defects. In our study, after we excluded NTD cases with known causes, our findings agreed with those of Holmes on the sibling precurrence rates for NTDs. However, both this study and that of Holmes are based on small numbers and need to be confirmed by larger studies.

A prominent finding in our study is the higher precurrence rate for birth defects in general among siblings of singles compared with multiples. Although our num-

TABLE 5

PRECURRENCE RATES OF BIRTH DEFECTS AMONG PARENTAL SIBLINGS OF NTD INDEX CASES, BY CATEGORY OF MALFORMATION: COMPARISON WITH MACDP POPULATION RATES

	Singles	Multiples	MACDP
Maternal siblings	(No. = 715)	(No. = 200)	...
NTDs	0.7%* (5)†	0.5% (1)	0.18%†
Other major defects	2.5% (18)	3.0% (6)	3.5%
All major defects	3.2%‡ (23)	3.5% (7)	3.7%
Paternal siblings	(No. = 692)	(No. = 174)	...
NTDs	0.0% (0)	0.0% (0)	0.18%
Other major defects	1.3% (9)	1.1% (2)	3.5%
All major defects	1.3%‡ (9)	1.1% (2)	3.7%

* Comparison of NTD rates with MACDP rate, $P = .0107$ (cumulative Poisson distribution).

† No. cases in parentheses.

‡ Comparison of rates of defects between maternal and paternal siblings among singles, $\chi^2 = 5.81$, $P = .0159$.

bers were small and statistical significance was reached only for all birth defects combined, the difference between the two groups was consistent for NTDs and for other major and minor defects. This finding is to our knowledge a new one, and there is no reason to believe that it is due to a difference in recall between mothers of singles and multiples. Besides, some of these cases were independently ascertained from the surveillance system. Two recent studies [11, 12] found a higher than expected occurrence of NTDs among siblings of children with tracheoesophageal dysraphism. These studies and our present one seem to suggest that mothers of babies with one birth defect may be at an increased risk for having babies with other types of birth defects, an important issue in both etiologic studies of birth defects and in genetic counseling.

Furthermore, our data suggest that single and multiple NTDs are etiologically different. In [1], we found that singles had declining rates over time, suggesting important environmental influences in their genesis. Our present study lends further support to this idea. The finding of an increased risk of birth defect for siblings born within a short time before the birth of the index case points more to important environmental effects operating during that time than to genetic factors (such as polygenic inheritance [13]), which would be randomly distributed. It is unlikely that a maternal recall bias accounts for this difference, since the miscarriage rates and precurrence rates of minor defects did not manifest similar trends. Yen and McMahon [14] found that the recurrence rates for NTDs following the birth of an affected child decreased somewhat over time. They suggested that this is evidence more in favor of important environmental determinants of NTDs than it is of genetic components.

At any rate, both genetic and environmental components may be interacting in producing the observed increased sibling risk for birth defects. It may be more beneficial not to view these two factors as mutually exclusive, as has long been the case in the issue of nature vs. nurture [15]. The recent studies of Embury et al. and Seller et al. [16, 17] may help to show the extent of this interaction in the pathogenesis of NTDs. The curly-tail mouse is a mutant strain that has a high rate of NTDs (mostly in females). Moreover, compared with other strains of mice, curly-tail mice showed an enhanced susceptibility to the teratogenic effect of vitamin A; more NTDs were produced if vitamin A was administered at the time of closure of the neural tube. Could curly-tail mice serve as a model for the occurrence of single NTDs in humans? Could mothers of infants with single NTDs be genetically predisposed to the action of one or more teratogens that produce not only NTDs but also other birth defects in their fetuses? These ideas are entirely speculative at this stage.

On the other hand, our data indicate that multiple NTDs may have a low sibling rate for NTDs and other birth defects. This finding is consistent with that of Holmes and, if confirmed by larger data sets, seems to indicate that the occurrence of multiple NTDs is a sporadic event, the cause of which could be genetic or environmental, most likely the latter. Further studies are needed to delineate the heterogeneity of multiple NTDs, which could be based on the types of the associated defects.

The difference in precurrence rate for NTDs between maternal and paternal siblings has been noted [18]. In our study, it probably reflects a more accurate history obtained from the mother about her side of the family compared with the father's. In support of this idea, in our study, paternal siblings were found to have a lower occurrence of major birth defects and of a variety of common diseases compared with maternal siblings.

Our approach of examining familial risks for birth defects according to the presence of associated anomalies has rarely been used. Erickson [4] found, among a group of selected birth defects that included NTDs, a higher precurrence rate in singles compared with multiples. We suggest that this approach be used more often in family studies of birth defects and also in searching for suspected risk factors. Our data may have an impact on family counseling if further studies show different recurrence risks between the two groups of NTDs.

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REFERENCES

1. KHOURY MJ, ERICKSON JD, JAMES LM: Etiologic heterogeneity of neural tube defects: clues from epidemiology. *Am J Epidemiol* 115:538–548, 1982
2. ELWOOD JM, ELWOOD JH: *Epidemiology of Anencephalus and Spina Bifida*. Oxford, Oxford Univ. Press, 1980, pp 85–138
3. EDMONDS LD, LAYDE PM, JAMES LM, FLYNT JW, ERICKSON JD, OAKLEY GP: Congenital malformations surveillance: two American systems. *Int J Epidemiol* 10:247–252, 1981
4. ERICKSON JD: Racial variation in the incidence of congenital malformations. *Ann Hum Genet* 39:315–320, 1976
5. ELWOOD JM, ELWOOD JH: *Epidemiology of Anencephalus and Spina Bifida*. Oxford, Oxford Univ. Press, 1980, pp 225–235
6. JANERICH DT, PIPER J: Shifting genetic patterns in anencephaly and spina bifida. *J Med Genet* 15:101–105, 1978
7. MCBRIDE ML: Sib risks of anencephaly and spina bifida in British Columbia. *Am J Med Genet* 3:377–387, 1979
8. NEVIN NC, JOHNSTON WP: A family study of spina bifida and anencephalus in Belfast, Northern Ireland (1964 to 1968). *J Med Genet* 17:203–211, 1980
9. CARTER CO, DAVID PA, LAURENCE KM: A family study of major central nervous system malformations in South Wales. *J Med Genet* 5:81–106, 1968
10. HOLMES LB, DRISCOLL SG, ATKINS LA: Etiologic heterogeneity of neural tube defects. *N Engl J Med* 294:365–369, 1976
11. DAVID TJ, O'CALLAGHAN SE: Esophageal atresia in the South West of England. *J Med Genet* 12:1–11, 1975
12. FRASER FC, NUSSBAUM E: Neural tube defects in sibs of children with tracheo-esophageal dysraphism. *Lancet* ii:807, 1980
13. CARTER CO: Genetics of common disorders. *Br Med Bull* 25:52–57, 1969
14. YEN S, MCMAHON B: Genetics of anencephaly and spina bifida? *Lancet* ii:623–662, 1968
15. HARRIS H: Nature and nurture. *N Engl J Med* 25:1399–1400, 1977
16. EMBURY S, SELLER MJ, ADINOLFI M, POLANI PE: Neural tube defects in curly-tail mice: 1. Incidence, expression and similarity to the human condition. *Proc R Soc Lond [Biol]* 206:85–94, 1979

17. SELLER MJ, EMBURY S, POLANI PE, ADINOLFI M: Neural tube defects in curly-tail mice. II. Effect of maternal administration of vitamin A. *Proc R Soc Lond [Biol]* 206:95–107, 1979
18. CARTER CO, EVANS K: Spina bifida and anencephalus in Greater London. *J Med Genet* 10:209–234, 1973

APPENDIX

BIRTH DEFECTS AMONG SIBLINGS OF NTD INDEX CASES, BY CATEGORY OF MALFORMATION

Birth defect categories	No. affected sibs	Index cases, by type of NTD and category of malformation
I. Neural tube defects:		
Anencephaly	2	2 SB * (S)†
Spina bifida	2	1 A (S)‡ 1 SB (S)
II. Other major defects:		
Microcephaly	1	1 A (S)
Ventricular septal defect	1	1 A (S)‡
Patent ductus arteriosus	1	1 SB (S)
Cleft lip/palate	2	2 A (S)
Clubfoot	1	1 SB (S)
Rectal stenosis	1	1 SB (S)
Pyloric stenosis	1	1 SB (M)
Down syndrome	1	1 SB (S)
Moebius syndrome	1	1 SB (S)
Chordee without hypospadias	1	1 SB (S)
III. Minor anomalies:		
Spina bifida occulta	1	1 SB (S)
Single umbilical artery	1	1 E (M)
Hydrocele	2	2 SB (S)
Inguinal hernia	1	1 SB (S)
Umbilical hernia	2	2 A (S)
Undescended testicle	1	1 SB (S)
Nasolacrimal duct obstruction	2	1 A (S), 1 SB (S)

* SB = spina bifida, A = anencephaly, E = encephalocele.
† (S) = single, (M) = multiple.
‡ One sibling had spina bifida and ventricular septal defect.