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ETIOLOGIC HETEROGENEITY OF NEURAL TUBE DEFECTS: CLUES FROM EPIDEMIOLOGY[1]

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Khoury, M. J. (Birth Defects Branch, CDC, Atlanta, GA 30333), J. D. Erickson and L. M. James. Etiologic heterogeneity of neural tube defects: clues from epidemiology. *Am J Epidemiol* 1982;115:538-48.

The epidemiology of neural tube defects was reviewed, using data from two birth defects surveillance systems: the nationwide Birth Defects Monitoring Program and the Metropolitan Atlanta Congenital Defects Program, for 1970–1978 and 1968–1979, respectively. After excluding cases with recognized causes, neural tube defects were divided into two major groups: "singles" and "multiples," depending on the presence of associated major defects. Only singles, which accounted for the majority of cases, were shown to have the well-known epidemiologic characteristics of neural tube defects: marked predominance of females and whites, geographic variation with an east-to-west gradient, and decreasing rates over time. On the other hand, 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. The presence of associated defects indicates that neural tube defects are epidemiologically and probably etiologically heterogeneous. It is suggested that analytic studies of neural tube defects may be more rewarding if they try to identify different risk factors associated with various subgroups. This approach to the study of birth defects may provide better clues to their etiology and pathogenesis.

abnormalities, multiple; classification; neural tube defects

Different congenital malformations have been reported to be associated with neural tube defects (1, 2), mostly in autopsy studies (3, 4). Some of these defects are secondary to the neural anomalies (e.g., adrenal hypoplasia), but others (e.g., cardiac defects) cannot be directly related. The presence and types of these associated defects may indicate that neural tube defects have multiple causes. Holmes et al. (5) found that the 12 per cent of their neural tube defect cases with known causes had other associated defects and had different sibling risks from the rest. Furthermore, they showed that among cases with no recognized causes, earlier-born siblings of cases with isolated neural tube defects had a higher rate of neural tube defects than siblings of cases with associated malformations (1.7 vs. 0.0 per cent). Holmes et al. suggested that the cause of neural tube defects in combination with other malformations differs from that of isolated ones. However, despite the possible heterogeneity, neural tube defects have almost always been grouped together in both descriptive and analytic epidemiologic studies. Despite

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Abbreviation H-ICDA, Hospital Adaptation of the *International Classification of Diseases, Adapted.*

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the wealth of available data on the subject (6), few attempts have been made to examine the epidemiologic characteristics of different groups of neural tube defects on the basis of presence of associated defects (7). This may be one of the reasons why the causation of neural tube defects remains elusive and their epidemiology puzzling (8).

With this background in mind, we reviewed the epidemiology of neural tube defects, using data from two birth defect surveillance systems: the nationwide Birth Defects Monitoring Program and the Metropolitan Atlanta Cogenital Defects Program. A small proportion of reported cases had recognized causes. The rest were classified into "single" and "multiple" categories, depending on the presence of associated nonsecondary major defects. The epidemiologic characteristics of these groups were compared and shown to be different.

Materials and methods

The Birth Defects Monitoring Program, a nationwide birth defects surveillance system, is a collaborative effort involving the Centers for Disease Control (CDC) and the Commission on Professional and Hospital Activities. The system is not population-based but covers approximately one million liveborn and stillborn infants per year widely distributed over the United States. Cases are ascertained from hospital discharge diagnoses from approximately 1200 hospitals. There is usually no attempt to follow up cases. For a selected group of birth defects (that include anencephaly and spina bifida but not encephalocele), information on individual cases is available to CDC via a periodical report from the Commission. All discharge diagnoses are coded by the system of the Hospital Adaptation of the International Classification of Diseases, Adapted (H-ICDA) (9, 10).

The Metropolitan Atlanta Congenital Defects Program, a birth defects surveillance system covering the five-county central Metropolitan Atlanta area, is a joint endeavor of CDC, the Georgia Mental Health Institute, and Emory University School of Medicine. The system operates independently from the Birth Defects Monitoring Program and covers approximately 24,000 births per year. Cases include all birth defects among stillborn and liveborn infants diagnosed in the first year of life. They are ascertained by several methods, which include review of hospital records of mothers and infants, cytogenetic laboratory results, and vital records. Because of adequate follow-up, we believe ascertainment of cases is essentially complete. The two surveillance systems have been described elsewhere (11, 12).

We reviewed available data on neural tube defects in the two systems. These included cases of anencephaly, spina bifida (meningocele, meningomyelocele), and encephalocele in the Atlanta program from 1968 to 1979, and cases of anencephaly and spina bifida only in the nationwide program from 1970 to 1978. We attempted to identify cases that had recognized causes (chromosomal anomalies, intrauterine infections, single gene disorders, and known teratogens). For that purpose, we had to rely on the clinical diagnosis in the record. In the Atlanta program, records of cases that had associated defects were reviewed, and if they had several features of known syndromes associated with neural tube defects, cases were labeled as such even if such diagnoses were not explicitly mentioned. In the nationwide program, no similar records could be reviewed, since only H-ICDA discharge diagnoses codes were available. In many instances, a birth defect code does not represent a specific entity but instead covers a group of conditions. After we excluded cases with recognized causes, the rest were classified into two major groups: 1) "singles" if there were no associated malformations or only

defects considered minor or secondary to neural tube defects, and 2) "multiples" if there were additional major defects. (Secondary defects included: other neural tube defects or central nervous system defects, clubfoot, congenital hip dislocation, flexion contractures, arthrogryposis, spine, rib and sternum defects, adrenal, thymus and lung hypoplasia. Minor defects were: slanting of the eyes, epicanthal folds, esotropia, nystagmus, lacrimal duct obstruction, small palpebral fissures, ear tags, low-set ears, branchial cleft, beaked nose, deviated septum, cleft gum, mucocele, ranula, natal tooth, tongue tie, high-arched palate, facial palsy, clinodactyly, camptodactyly, simian creases, broad phalanges, rocker-bottom feet, plantar furrow, overlapping toes, pigmented nevus, pilonidal sinus, scalp defect, scars, accessory nipples, skin tags, hydrocele, undescended testicle, hymenal and vaginal tag; all hernias except diaphragmatic.)

Results

In the nationwide program, 9403 cases were ascertained in the period 1970–1978; anencephaly and spina bifida had rates of 0.5 and 0.6 per 1000 live births, respectively. In the Atlanta program, 563 cases were ascertained in the period 1968–1979. Both anencephaly and spina bifida had higher rates in the Atlanta program than in the nationwide program (0.7 and 0.9 per 1000 live births, respectively). Fifty-two cases of encephalocele were ascertained through the Atlanta program (rate of 0.2 per 1000 live births).

Classification of neural tube defects

Very few cases in the two systems (<1 per cent) could be attributed to recognized causes; most of these had chromosomal anomalies (table 1). Of the four cases of Meckel's syndrome detected in the Atlanta program, only one was labeled as such in the record. Also detected in that program was one case of fetal alcohol syndrome associated with spina bifida, a combination previously reported (13).

TABLE 1

Recognized causes of neural tube defects in the Birth Defects Monitoring Program (BDMP), 1970–1978, and the Metropolitan Atlanta Congenital Defects Program (MACDP), 1968–1979

Causes	BDMP* (*n* = 9403)	MACDP† (*n* = 563)
Chromosomal anomalies	46	1
Down syndrome	14	
Other autosomal anomalies		
Trisomy 18	12	
Trisomy 13	4	
Not specified	10	1
Sex chromosomal anomalies	6	
Intrauterine infections	5	
Congenital syphilis	3	
Congenital rubella	1	
Congenital toxoplasmosis	1	
Genetic disorders	5‡	4§
Teratogens		1¶
Total	56 (0.6%)	6 (1.1%)

* Includes anencephaly and spina bifida.
† Includes anencephaly, spina bifida, and encephalocele
‡ H-ICDA code 759.8
§ Meckel's syndrome (4 cases).
¶ One case of fetal alcohol syndrome.

The vast majority of cases were divided into singles and multiples according to the above criteria. Table 2 shows that 88 per cent of cases in the nationwide program and 80 per cent in the Atlanta program belonged to the single category. Both anencephaly and spina bifida had a higher proportion of multiples in the Atlanta program. Of the major groups of malformations associated with multiple neural tube defects (table 3), almost all tended to occur more often in cases ascertained through the Atlanta program.

Epidemiologic characteristics of singles and multiples

Race and sex. In the two systems, singles and multiples had different race- and

TABLE 2

Neural tube defects by category of malformation, Birth Defects Monitoring Program (BDMP), 1970–1978 and the Metropolitan Atlanta Congenital Defects Program (MACDP), 1968–1979

Defects	BDMP (n = 9347)		MACDP (n = 557)	
	Single	Multiple	Single	Multiple
Anencephaly	89.8% (3603)*	10.2% (411)	82 1% (183)	17.9% (40)
Spina bifida	86.3% (4603)	13.7% (730)	79.0% (226)	21.0% (60)
Encephalocele			71.9% (35)	27 1% (13)
All	87.8% (8206)	12.2% (1141)	79 7% (444)	20.3% (113)

* Number of cases in parentheses.

sex-specific rates (figures 1 and 2). Singles occurred predominantly in whites, with sex-adjusted relative risks of 2.8 for Atlanta and 1.8 nationwide. Multiples had a lesser but statistically significant predominance of whites over other races (relative risks of 1.9 and 1.4, respectively). Singles occurred mostly in females (especially white females) with race-adjusted relative risks of 1.8 for Atlanta and 1.5 nationwide; multiples occurred equally in both sexes (relative risks of 1.1 and 0.9, respectively). These findings were noted for both anencephaly and spina bifida. Encephalocele had similar trends, but the numbers were too small for meaningful comparison.

Secular trends. As seen in figures 3 and 4, rates of singles and multiples had different trends over time. In both systems, singles had significantly declining rates (annual per cent declines of 5.6 nationwide and 8.3 for Atlanta, both slopes of regression lines being significantly different from 0, $p < 10^{-6}$). These declines occurred in both anencephaly and spina bifida, in both sexes and both race groups. Although in the Atlanta program the rate of multiples did not change over time, in the nationwide program the rates decreased slightly (annual per cent decline 3.7, $p = 0.03$). This decrease, however, was significantly less than that of singles ($F = 28.59$, $p < 0.001$).

TABLE 3

Major groups of malformations associated with multiple neural tube defects in the Birth Defects Monitoring Program (BDMP), 1970–1978, and the Metropolitan Atlanta Congenital Defects Program (MACDP), 1968–1979

Defects	BDMP (n = 1141)		MACDP (n = 113)	
	No	%	No	%
Cardiovascular anomalies	133	11.7	26	23 0
Urinary tract anomalies	158	13.8	20	17.7
Cleft lip/palate	151	13.2	22	19.5
Omphalocele/gastroschisis	96	8.4	23	20.4
Limb reduction deformities	50	4.4	17	15.0
Polydactyly	40	3.5	8	7.1
Anorectal atresia	85	7.4	5	4.4

Geographic distribution. According to the nationwide data, the two groups had different geographic distributions. Singles manifested the east-to-west gradient characteristic of neural tube defects (6) (figure 5); (test for homogeneity, chi-square = 175.8, $p < 0.0001$), but multiples had relatively constant rates over the United States (chi-square = 11.7, $0.1 < p < 0.2$). These findings applied for both anencephaly and spina bifida.

Delivery outcome and mortality. In both systems, there was no appreciable difference in the proportion of stillbirths among singles and multiples. However, in the nationwide program, multiples were shown to have a higher neonatal mortality rate at hospital discharge than singles (52.3 vs. 38.6 per cent). In the Atlanta program, multiples had a higher infant mortality rate than singles (63.3 vs. 46.9 per cent). In the two systems, more autopsies

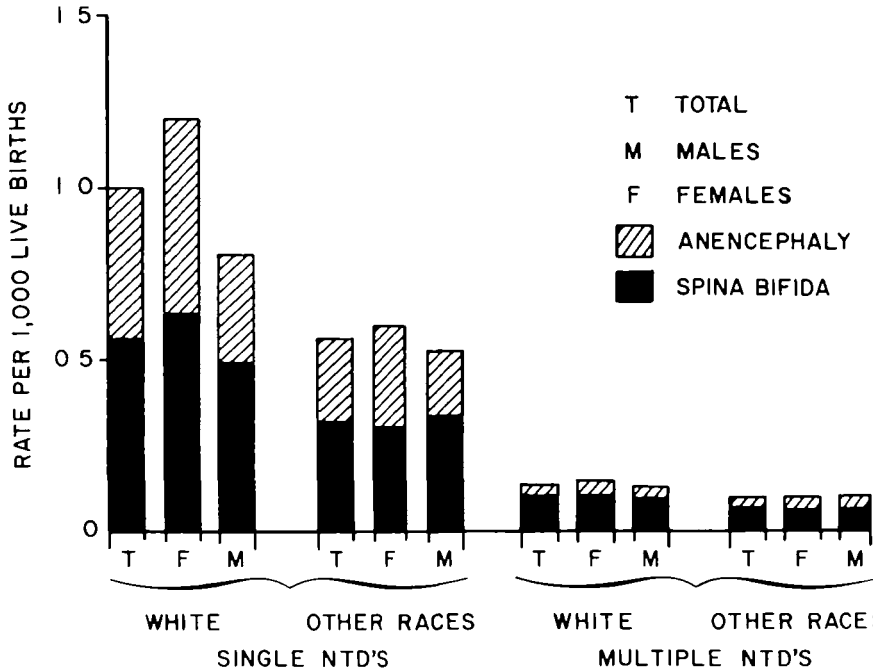

FIGURE 1. Neural tube defects (NTD) rates, by race, sex, and category of malformation, nationwide Birth Defects Monitoring Program, 1970–1978.

were done in multiples than in singles (nationwide: 56.9 vs. 27.6 per cent; Atlanta: 48.8 vs. 23.4 per cent).

Occurrence in twins. The proportion of twins among neural tube defect cases was generally higher in multiples in both systems (table 4). Moreover, more multiple twins were concordant for neural tube defects compared with singles (13.3 vs. 6.6 per cent in the nationwide program) (table 4). The sex ratios were markedly different in affected twins of both groups. Single twins had a predominance of females; multiple twins, males.

Other epidemiologic variables. No difference was found between singles and multiples with respect to other epidemiologic indices, including maternal age, parity, season of birth, birth weight, and gestational age.

DISCUSSION

These results indicate that neural tube defects are an epidemiologically heterogeneous group of conditions, depending on the presence of associated defects. The epidemiologic characteristics of neural tube defects repeatedly described in the literature seem to apply only for cases with neural tube defects as the only major anomaly; these account for 80–88 per cent of all cases, depending on the surveillance system. These characteristics are marked predominance of females, excess of whites, geographic variation with east-to-west gradient, and decreasing rates over time. Neural tube defects that occur in combination with other birth defects have different epidemiologic characteristics: equal sex distribution, a lesser predominace of whites, no geographic variation, and little or no decrease in rates over time.

This study has several limitations. The first is that very few of our cases (<1 per cent) were reported to have known causes, compared, for example, with 12 per cent of neural tube defect cases described by

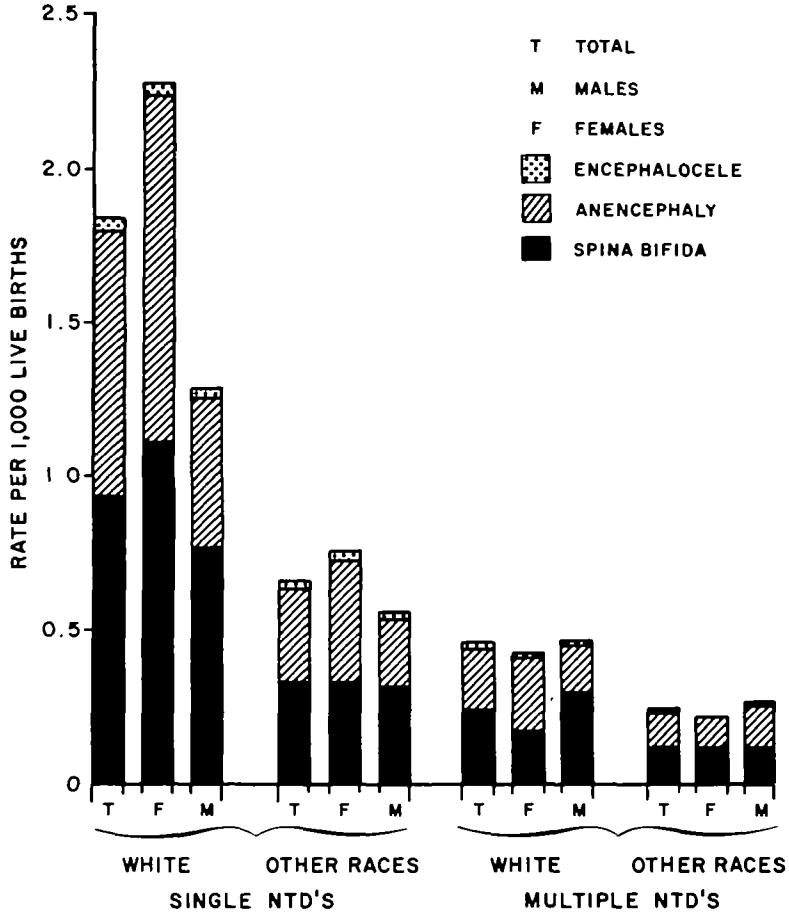

FIGURE 2. Neural tube defects (NTD) rates, by race, sex, and category of malformation, Metropolitan Atlanta, 1968–1979

Holmes et al. (5). In that study, half of these cases had recognized phenotypes of unknown etiology. In our study, such cases were not considered to have known causes, since their etiology is truly unknown. Furthermore, in the Holmes et al. study, almost all of the remaining cases with known causes were encephalocele with Meckel's syndrome. In our study, there were no encephalocele cases studied in the nationwide data, and of the 52 encephalocele cases in the Atlanta program, four (8 per cent) had Meckel's syndrome. Because we had to rely on the clinical diagnoses available in the records and because of the inadequacies of the H-ICDA coding system, we believe that our identification of cases with recognized causes is incomplete. This mostly applies to the many described syndromes with Mendelian inheritance.

The second problem concerns the validity of the classification of singles and multiples. Multiples had higher autopsy rates than singles. If more autopsies had been done, an unknown proportion of singles may have been identified as multiples. In an autopsy study, David and Nixon (3) found that 40 per cent of anencephalics and iniencephalics had other associated defects. To assess the extent to which autopsies affected our classification, we ex-

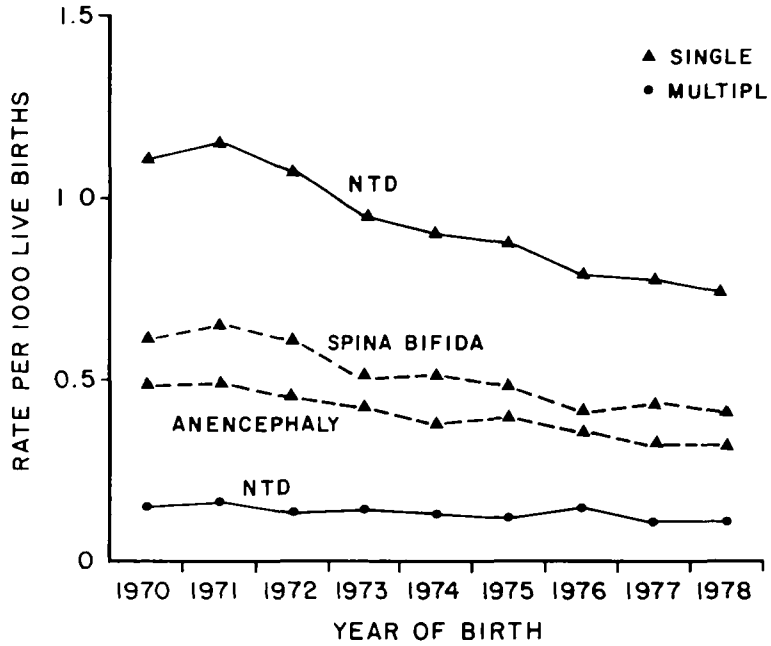

FIGURE 3. Neural tube defects (NTD) rates, by year of birth and category of malformation, nationwide Birth Defects Monitoring Program, 1970–1978

amined neural tube defect cases on whom autopsy information was available in the Atlanta program. Of 59 such cases, 39 had no internal defects added to the premortem clinical diagnosis. None of the 20 remaining cases that had more defects discovered on autopsy had been misclassified before the autopsy. For example, a single anencephalic was still classified as single after autopsy if only adrenal hypoplasia was discovered, and a multiple with limb defects remained classified as multiple if congenital heart disease was also discovered. Thus, our classification is valid at least in the Atlanta program, despite possible underreporting of individual defect groups.

The last problem concerns discrepancies in the results obtained from both systems. As noted earlier, the Atlanta program reported higher rates of neural tube defects, a larger proportion of multiples, and a higher frequency of individual birth defect categories associated with multiples, compared with the nationwide program. We believe that these discrepancies reflect the different methods of case ascertainment in the two systems. Despite these differences, however, the described epidemiologic characteristics and trends generally apply for neural tube defects in both systems. Furthermore, despite the relative underascertainment of multiples in the nationwide program, the geographic variation demonstrated for singles and not multiples is probably a true one since there is no reason to suspect a differential ascertainment of singles and multiples in different areas.

The epidemiologic heterogeneity of neural tube defects described in this study may reflect an underlying causal heterogeneity. Our data suggest at least two broad etiologic categories in the pathogenesis of neural tube defects. The first affects predominantly females and produces neural tube defects without associated malformations (singles); and the second affects both sexes equally and produces neural tube defects in association

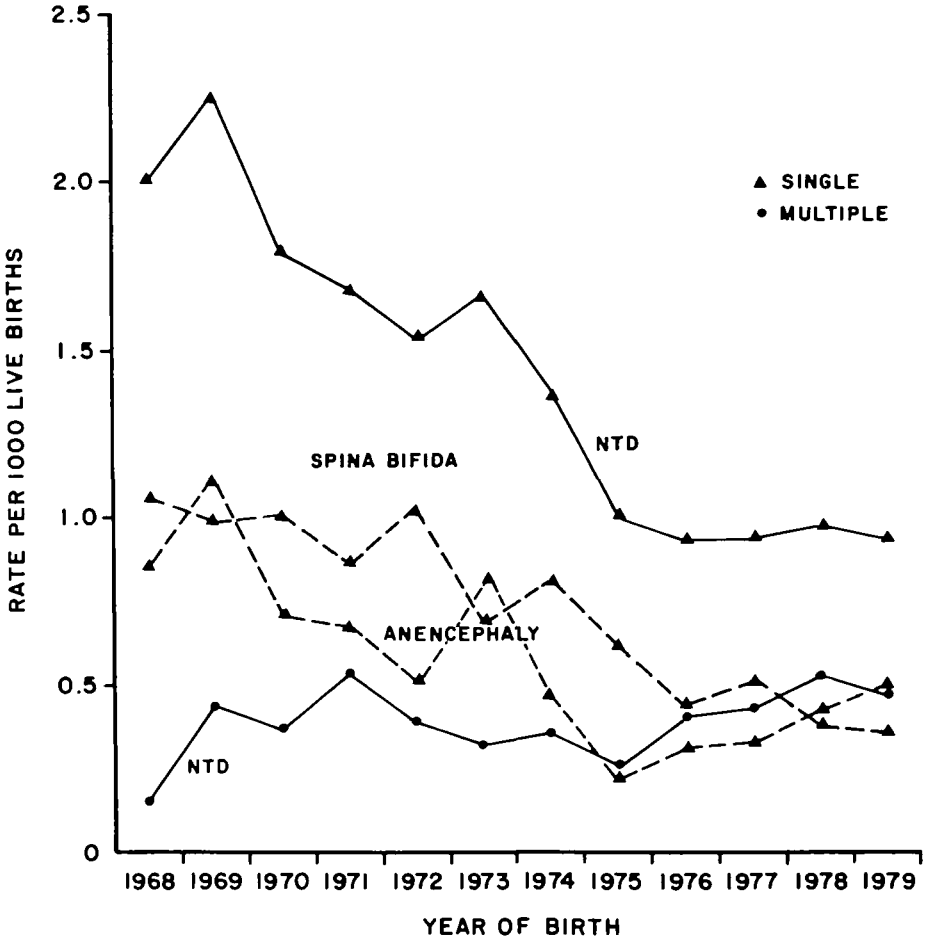

FIGURE 4 Neural tube defects (NTD) rates, by year of birth and category of malformation, Metropolitan Atlanta, 1968–1979

with other nonsecondary defects (multiples). Two causes of neural tube defects have already been postulated by James (14, 15). On the basis of the observation that anencephalic sex ratios vary with their prevalence at birth (with higher rates being associated with lower sex ratios), he postulated two causes of anencephaly: an environmental cause affecting female embryos and another, perhaps genetic, affecting both sexes. James' findings concerning sex ratio variation in anencephaly, however, could not be duplicated for spina bifida (16), which may have the same causal mechanism as anencephaly. Furthermore, his hypothesis does not consider associated defects. In our study, neural tube defects can be separated into two groups depending on the presence of associated defects; this distinction seems to apply for both anencephaly and spina bifida.

The epidemiologic features of single neural tube defects can be explained by genetic and/or environmental factors. A genetic hypothesis can explain the observed geographic variation by postulating greater outbreeding effects among

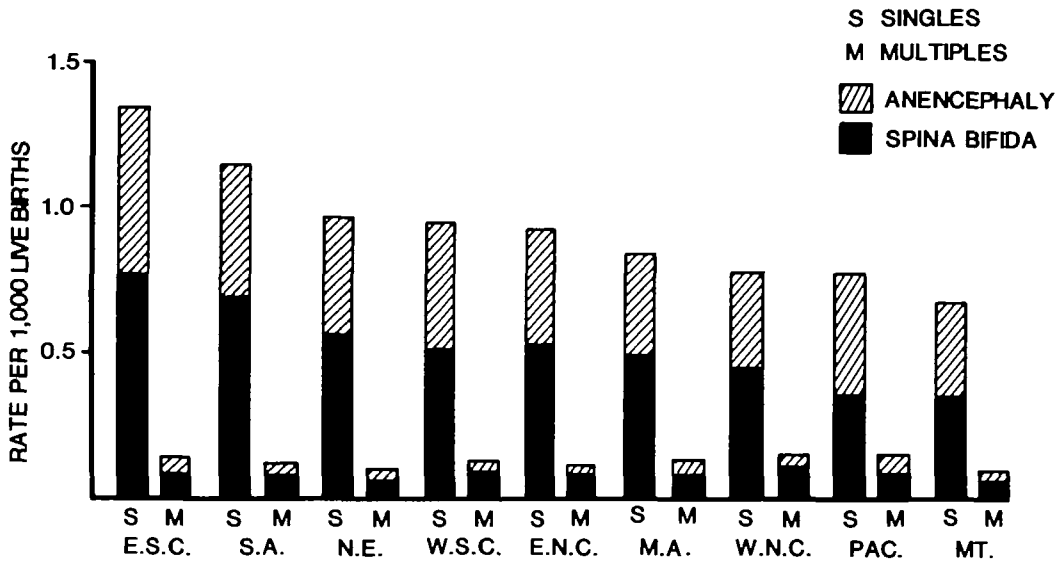

FIGURE 5. Neural tube defects rates, by US Census division and category of malformation, nationwide Birth Defects Monitoring Program, 1970–1978. Abbreviations. E.S C , East South Central; S.A., South Atlantic; N.E., New England; W S.C., West South Central; E N.C., East North Central, M A , Middle Atlantic, W.N.C , West North Central, PAC., Pacific; MT , Mountain.

Western inhabitants (17). Genetic changes may also be consistent with the decreasing rates of singles over time, especially since previous reports have suggested a long-term falling incidence of neural tube defects (18). Furthermore, one of us (JDE) has previously found among a group of birth defects (including neural tube defects) that earlier-born siblings of single cases had a higher rate than siblings of multiples (7). He suggested that genetic factors are more important in causing single birth defects. On the other hand, environmental differences can also explain the observed geographic variation. Moreover, since the falling incidence of neural tube defects has followed an apparent epidemic in the 1930s (18), an environmental hypothesis can more plausibly account for both the rise and decline in rates than a genetic theory. The present decline could be viewed as the descending limb of an epidemic curve. Several environmental factors could be mentioned as accounting for the declining rates of singles. These include withdrawal of unknown teratogens (e.g., drugs, dietary factors, etc.), increasing use of protective factors (e.g., vitamins) or an increasing interpregnancy gap as a result of more widespread use of contraceptives (19, 20). It is also worth mentioning that the decreasing rate of singles cannot be explained on the basis of increasing use of prenatal diagnosis, since prenatal screening has not been widely used so far, except in high risk pregnancies (positive family histories) which account for only a minority of cases (6). Lastly, the previously observed high sibling risk among singles can also be explained on the basis of common environmental factors operating in high risk families (18, 21). At any rate, both genetic and environmental factors may be operating in the production of single neural tube defects, and it is probably more beneficial not to view them as mutually exclusive components.

On the other hand, multiple neural tube defects are probably a heterogeneous group of conditions, depending on the na-

TABLE 4

Neural tube defects: occurrence in twins, twin concordance and sex ratios, by category of malformation, in the Birth Defects Monitoring Program (BDMP), 1970–1978, and the Metropolitan Atlanta Congenital Defects Program (MACDP), 1968–1979

	BDMP		MACDP	
	Singles	Multiples	Singles	Multiples
% twins	1.9%	2.5%	1.6%	5.0%
Observed	151‡	30§	7¦	6¶
Expected*	122.6	17.4	7.9	2.1
% concordant for neural tube defects	6.6% (10)†	13.3% (4)	0.0% (0)	16.7% (1)
Sex ratio of affected cases M/F)	0.69	1.73	0.75	1.00

* Expected figures are derived from rates in the two systems, adjusted for race
† Number of cases in parentheses
‡ $p = 0.07$ (Using cumulative Poisson distribution)
§ $p = 0.03$
¦ $p = 0.67$
¶ $p = 0.02$

ture of associated defect(s). A proportion of those cases may be caused by single autosomal gene disorders, which equally affect both sexes and would not show changes in rates over short periods.

A plausible explanation for different pathogenetic mechanisms between singles and multiples is that singles may be produced by failure of the neural tube to close; multiples may be produced by its rupture. The latter mechanism has long been postulated by Gardner (22), who recently suggested that overdistention of the neural tube may be a factor in the production of nonneural anomalies (23, 24). Although most investigators favor the hypothesis of initial failure to close (4), there is experimental evidence to support both mechanisms. Furthermore, many agents experimentally shown to produce overdistention of the neural tube have been associated with a variety of defects, in addition to neural tube defects (25, 26). Thus, although most neural tube defects may be caused by a primary failure of the neural tube to close, a proportion of cases may be caused by its rupture after primary closure. In these cases, other defects will be produced, depending on the teratogenic agent, timing of the rupture, and the anatomic locations involved.

The occurrence of neural tube defects found in twins in this study deserves further investigation. Among singles, 1.6 per cent and 1.9 per cent of all cases in the Atlanta program and the nationwide program, respectively, were twins. In addition, the twin concordance rate for neural tube defects was 6.6 per cent. These figures are comparable to those in Elwood and Elwood's recent update on the epidemiology of anencephaly and spina bifida (6). On the other hand, multiples had a higher proportion of twins (5.0 per cent (Atlanta) and 2.5 per cent (nationwide)) and a greater concordance for neural tube defects (13.3 per cent). These results support the idea that the fetus-fetus interaction model (27) may operate in the genesis of singles and not multiples. Further data are needed on the occurrence of neural tube defects in monozygotic and dizygotic twins according to associated defects.

We suggest that analytic studies should subdivide neural tube defects into subgroups in searching for causative agents. In these studies, grouping neural tube defects together may dilute or even conceal the effects of suspected risk factors if these apply only to a subgroup of neural tube defects. This would affect mostly multiples, since they account for only a minority of the cases. Furthermore, multiple neural tube defects may also be an etiologically heterogeneous group of conditions, depending on the type of the associated defect(s).

The approach to the study of birth defects by subdividing them according to the presence of associated defects has been previously used, though not fully explored. Erickson (7) found that, in a selected group of birth defects, singles and

multiples had different race-specific rates as well as different rates among earlier-born siblings. Emanuel et al. (28) found different maternal age distributions between single and multiple orofacial clefts. This approach may be more rewarding in elucidating the etiologic mechanisms of birth defects and may offer better understanding of their pathogenesis.

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