Neural Tube Defects and Associated Anomalies before and after Folic Acid Fortification

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Objective To examine the prevalence and types of neural tube defects and the types of anomalies co-occurring with neural tube defects in 6 years before fortification of cereal grain flour with folic acid (1992-1998) and 20 years after fortification (1999-2018) in South Carolina, a state with a historically high prevalence of these birth defects.

Study design The prevalence of neural tube defects was determined by active and passive surveillance methods in South Carolina since 1992. The types of neural tube defects and co-occurring malformations were determined by prenatal ultrasound and post-delivery examination.

Results In the 6 prefortification years, 363 neural tube defects were identified among 279 163 live births and fetal deaths (1/769), 305 (84%) of which were isolated defects of the calvaria or spine. In the 20 fortification years, there were significant reductions in the prevalence and percentage of isolated defects: 938 neural tube defects were identified among 1 165 134 live births and fetal deaths (1/1242), 696 (74.2%) of which were isolated. The current prevalence of neural tube defects in South Carolina (0.56/1000 live births and fetal deaths) is comparable with that nationwide.

Conclusions The continued occurrence of neural tube defects, the majority of which are isolated, after folic acid fortification of cereal grain flours suggests that additional prevention measures are necessary to reduce further the prevalence of these serious defects of the brain and spine. *(J Pediatr 2020;226:186-94)*.

Birth defects occur as isolated anomalies affecting a single anatomical structure and as more complex disorders in the company of anomalies in other portions of the anatomy.[1,2] Isolated birth defects far outnumber nonisolated birth defects affecting multiple anatomical systems. Among birth defects with other anomalies, there are those that have specific genetic or environmental causes, a number of clinically recognizable phenotypes for which specific causation is not known, and those that do not comprise clinically recognizable syndromes nor have a plausible genetic or environmental cause.

Most neural tube defects are isolated malformations of the central nervous system and its protective encasements. In surveys, 15%-25% of neural tube defects are accompanied by anomalies of other systems.[3-5] Enhancement of folic acid intake in the periconceptional period is a well-established method of lowering the risk of neural tube defects. This survey examines the neural tube defect prevalence and types of anomalies associated with neural tube defects in 6 prefortification years (1992-1998) and 20 fortification years (1999-2018) in South Carolina. Folic acid supplementation in the periconceptional period was the major prevention strategy during the prefortification years, whereas both supplements and fortification of cereal grain flours were used during the fortification years.[6]

Methods

The South Carolina Neural Tube Defects Prevention Program began on October 1, 1992, and has operated continuously. In the first year, surveillance and prevention activities covered 14 of the state's 46 counties and thereafter included all 46 counties. From 1992 to 2006, surveillance and prevention activities were conducted by the Greenwood Genetic Center. Neural tube defects were identified through the state's 3 genetics referral services and through all the state's prenatal diagnostic centers, obstetrician offices, maternal alpha-fetoprotein laboratories, and delivery hospital medical records. In 2006, surveillance was transferred to the South Carolina Department of Health and Environmental Control and uses hospital medical records as the major source of case identification. Prevention activities continued to be conducted by the Greenwood Genetic Center.

The South Carolina Neural Tube Defects Prevention Program followed the recommendation made in 1992 by the US Centers for Disease Control and Prevention for increased folic acid use (400 μg/d) for all women of childbearing years and the 1991 recommendation for women with a previous affected pregnancy to use 4000 μg/d in the periconceptional period to prevent recurrences.[7,8] In addition, enriched cereal grain products in the state were fortified beginning in 1998 as mandated by the US Food and Drug Administration.[9]

Details collected on the neural tube defect–affected pregnancy, fetus, or infant included sex, race, nature of the neural tube defect (location, and isolated or

OEIS Omphalocele, Exstrophy, Imperforate anus, Sacral anomalies

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Supported by the South Carolina Department of Disabilities and Special Needs (2020-43 [to R.S.]). The authors declare no conflicts of interest.

https://doi.org/10.1016/j.jpeds.2020.07.002

nonisolated), pregnancy complications or exposures, gestational age at delivery, laboratory test results, surgical procedures, and current status of liveborn infants. The number of live births, fetal deaths, and terminations each year were obtained from South Carolina Vital Records.

Women with neural tube defect–affected pregnancies were offered enrollment in a neural tube defect recurrence prevention program. Those enrolled were consented using the form reviewed and approved by the Self Regional Healthcare institutional review board. Enrollees were contacted every 3 months to reinforce prevention information and every month when a pregnancy occurred.

Terms Used

Anencephaly, spina bifida, and encephalocele are the 3 categories of neural tube defects used by the South Carolina Neural Tube Defects Prevention Program. If 2 defects are present, the case is included in the more severe category. Iniencephaly (n = 8) is included in the anencephaly category because of defects in the calvaria and spine. Cutis aplasia of the scalp is included in the encephalocele category. The single case of multiple vertebral anomalies is included as spina bifida.

Isolated neural tube defects have 1 of the 3 types of neural tube defects and no other major malformation except those that are considered secondary to the neural tube defects (hydrocephalus, tethered cord, omphalocele, or club foot). Nonisolated neural tube defects include 3 categories: Category I: Nonisolated neural tube defects with specific genetic or environmental causes known include neural tube defects with other birth defects and known causes. The genetic causes (Category Ia) include chromosomal aberrations, microduplications, microdeletions, and single-gene disorders. Environmental influences (Category Ib) include maternal diseases, obesity, and pregnancy exposures; Category II: Nonisolated neural tube defects with clinically recognizable phenotypes include neural tube defects with other birth defects that constituted a recognizable pattern of malformation but with cause unknown; Category III: Nonisolated neural tube defects with nonrecognizable phenotype include neural tube defects with other birth defects that do not constitute a recognizable pattern of malformation and which do not have a specific known cause.

Rates of neural tube defects are given as rates per 1000 live-births and fetal deaths. Pregnancy terminations are not included in these numbers.

Folate-resistant neural tube defects are those neural tube defects that occurred even though the mother was taking folic acid supplements in the periconceptional period (prefortification years) or consumed fortified food products with or without supplements in the periconceptional period (fortification years). Periconceptional use of folic acid supplements indicates intake for at least 1 month before conception and 1 or more months following conception.

Statistical Analyses

The Fisher exact test was used to test the effect of fortification on isolated and complex cases separately for all neural tube defects, spina bifida, anencephaly, and encephalocele cases. OR, CIs, and *P* values were calculated using the Baptista–Pike method using GraphPad Prism 8.3.1 (GraphPad Software, San Diego, California).

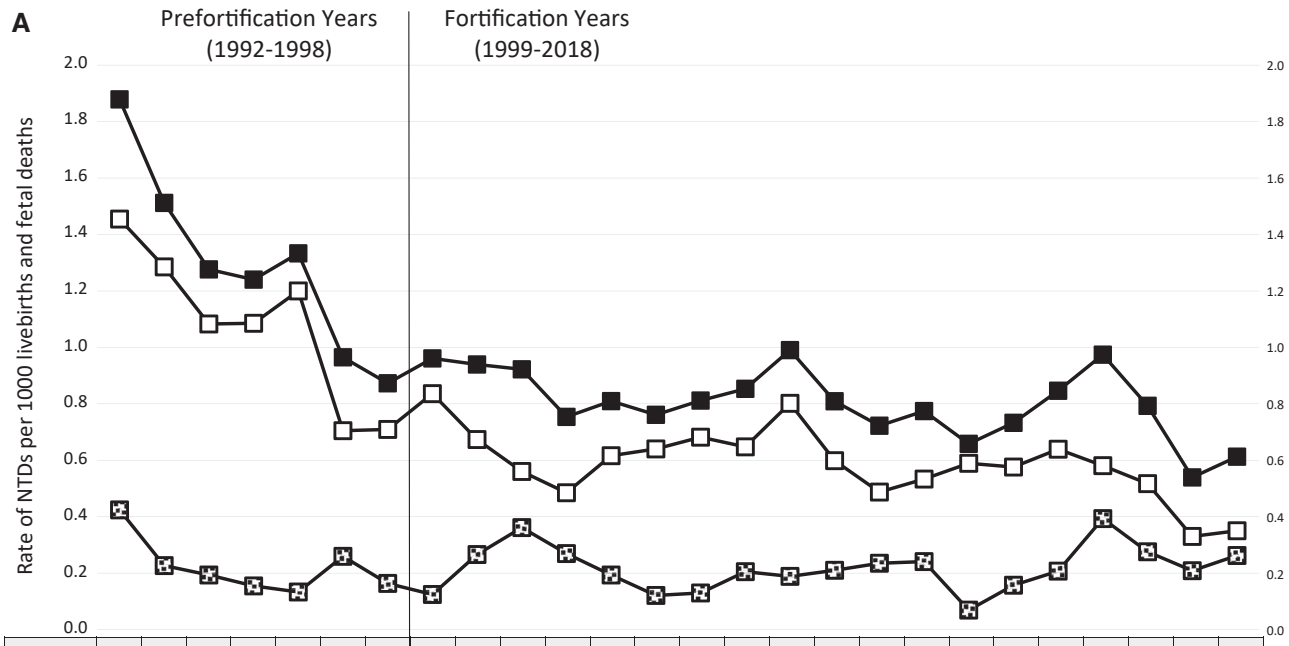
Results

All Surveillance Years

Since initiation of surveillance for neural tube defects and use of folic acid prevention/treatment strategies in 1992, the overall prevalence of neural tube defects in South Carolina has decreased from 1.87 cases to 0.56 cases per 1000 live births and fetal deaths, which includes spontaneous fetal deaths, but does not include pregnancy terminations. During this 26-year period, 1301 neural tube defect cases were identified. During the prefortification years (1992-1998), live-births accounted for 151 (41.6%) of cases, terminations for 180 (49.6%), and fetal deaths for 32 (8.8%). During the fortification years (1999-2018), live births accounted for 475 (50.6%) cases, terminations for 299 (31.9%), and fetal deaths for 161 (17.2%). The source of ascertainment was unknown for 3 cases in the fortification years (**Table I**; available at www.jpeds.com). The 1301 neural tube defects identified in the prefortification and fortification years included 466 cases of isolated spina bifida and 128 cases of nonisolated spina bifida, 416 cases of isolated anencephaly and 89 cases of nonisolated anencephaly, and 119 cases of isolated encephalocele and 83 cases of nonisolated encephalocele (**Figure 1** and **Table II** [**Table II** available at www.jpeds.com]). The type of neural tube defect and the presence of other anomalies was determined by prenatal ultrasonography only (202, 15.5%), postnatal examination (931, 71.6%), and autopsy (168, 12.9%). The year-by-year prevalence rates are shown in graphic form in **Figure 1**. Recurrences accounted for only 18 (1.4%) of the cases.

Genetic laboratory testing was performed on a minority of the 1301 neural tube defects. Chromosomal analysis was completed on 405 cases (121 prefortification), microarray analysis for microduplications and microdeletions on 36 cases (6 prefortification), whole-exome sequencing on 39 cases (13 prefortification), and whole-genome sequencing on 58 cases (23 prefortification).

In South Carolina, similar to other states, Hispanic subjects had the greatest prevalence of neural tube defects, white subjects an intermediate prevalence, and black subjects the lowest prevalence. The prevalence of isolated neural tube defects among Hispanic subjects was 0.87 in 1000 live births and fetal deaths, among white subjects 0.78 in 1000, and among black subjects 0.49 in 1000. The prevalence for nonisolated neural tube defects was 0.29 in 1000 live births and fetal deaths for Hispanic subjects, 0.20 in 1000 for white subjects, and 0.20 in 1000 for black subjects. The prevalence in the prefortification and fortification years by race is provided in **Table III** (available at www.jpeds.com). The male/female ratio for all neural tube defects was 0.9 for the 1144 cases in



Project Year	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
All NTDs	1.88	1.51	1.28	1.24	1.33	0.96	0.87	0.96	0.94	0.92	0.75	0.81	0.76	0.81	0.85	0.99	0.81	0.72	0.77	0.66	0.73	0.85	0.97	0.79	0.54	0.61
Isolated NTDs	1.45	1.29	1.08	1.09	1.20	0.71	0.71	0.84	0.67	0.56	0.48	0.62	0.64	0.68	0.65	0.80	0.60	0.49	0.53	0.59	0.58	0.64	0.58	0.52	0.33	0.35
With Anomalies	0.42	0.23	0.19	0.16	0.13	0.26	0.16	0.12	0.27	0.36	0.27	0.19	0.12	0.13	0.21	0.19	0.21	0.24	0.24	0.07	0.16	0.21	0.39	0.28	0.21	0.26
Livebirths and Fetal Deaths	16.5	52.9	51.7	51.6	52.5	53.9	55.0	56.2	56.4	55.3	55.7	56.8	57.8	61.6	63.3	63.6	61.8	59.5	58.1	57.7	57.3	57.9	58.5	58.0	57.5	57.1

Figure 1. **A,** Year-by-year prevalence for all isolated and nonisolated neural tube defects. **B,** Year-by-year prevalence for isolated and nonisolated spina bifida, anencephaly, and encephalocele cases. Annual deliveries (live births and fetal deaths) are provided at the *bottom* of **A**. Note the scale change in the encephalocele rates. *(Continues)*

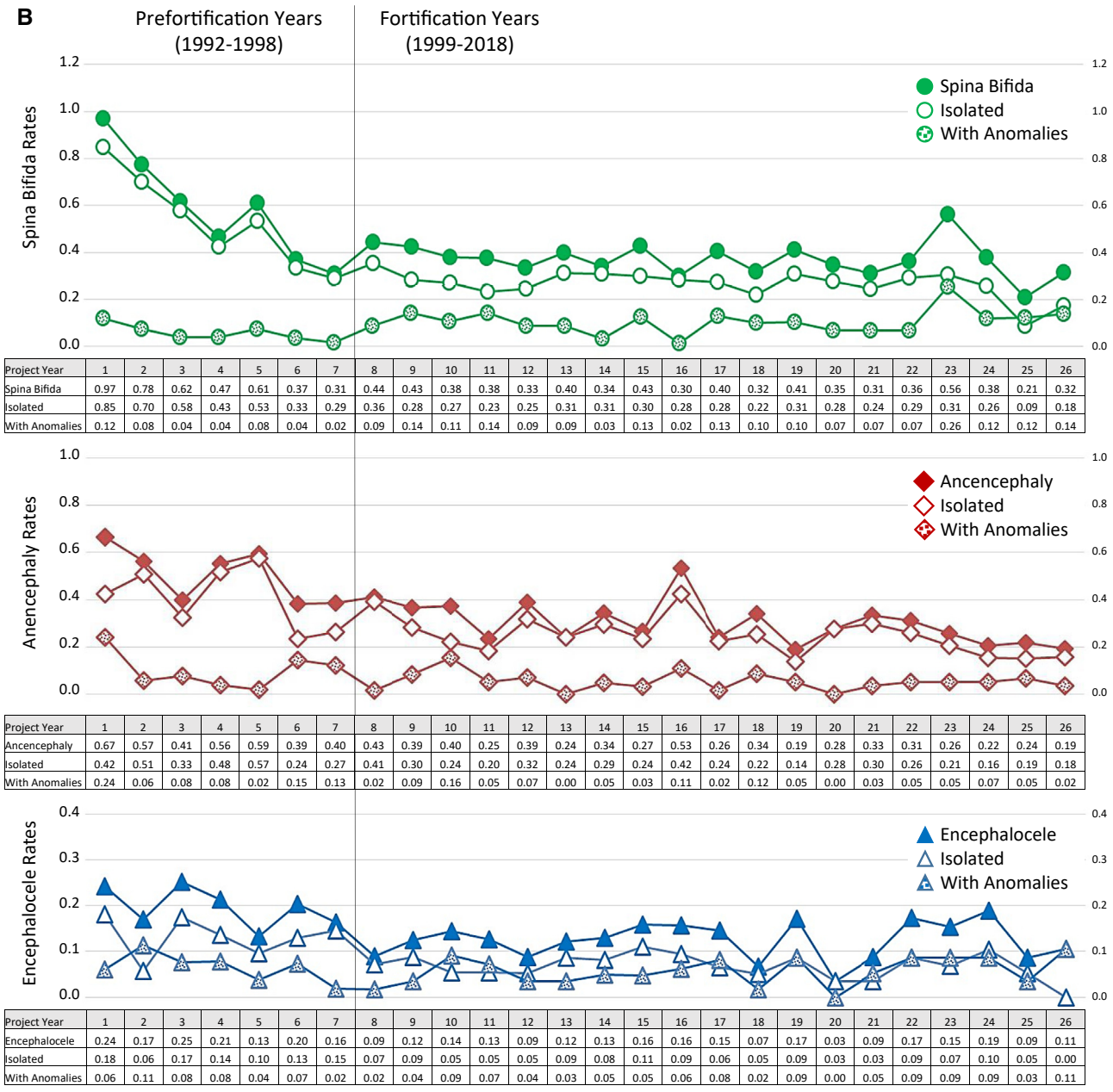
which the sex was known. The male/female ratio was 1.05 among spina bifida cases, 0.7 among anencephaly cases, and 0.94 among encephalocele cases.

Of the 1301 neural tube defects identified in the 26-year period (1992-2018) in South Carolina, 300 (23%) had additional anomalies (**Figure 1** and **Table II**). A specific cause or a clinically recognizable pattern of malformations (Category Ia, Ib: nonisolated neural tube defects with specific cause known and Category II: non-isolated neural tube defects with clinically recognizable phenotype of unknown cause) could be identified in slightly more than one-half (161/300; 53.7%) of instances in which neural tube defects were associated with other anomalies (**Figure 2** and **Table IV** [Table IV available at www.jpeds.com]). Chromosomal aberrations (n = 77), single-gene disorders (n = 19), and prenatal environmental influences (n = 1) were the specific causes found among this group. Among the chromosomal aberrations, trisomy 18 was found in 40 cases (32 spina bifida, 4 anencephaly, and 4 encephalocele), trisomy 13 in 8 cases (5 spina bifida and 3 encephalocele), and triploidy in 8 cases (all spina bifida). Other trisomies (4), mosaic trisomies (3), and chromosomal translocations, duplications, deletions, or markers (14) accounted for the remaining chromosomally abnormal cases (**Table V**). Single-gene disorders included Meckel syndrome (18 cases, 7 in prefortification years, 11 in fortification years), and 1 *CASP9* mutation (in fortification years). A single case of prenatal methimazole exposure was included in Category Ib (in fortification years).

Neural tube defects with clinically recognizable phenotypes of unknown cause (Category II) included amniotic bands syndrome (43/300, 14.3%), OEIS complex, ie, Omphalocele, Exstrophy, Imperforate anus, Sacral anomalies (12/300, 4%), hemifacial microsomia (3/300, 1%), sirenomelia (3/300, 1%), renal and Müllerian dysgenesis (3/300, 1%), and short cord syndrome (2/300, 0.7%). One case with sirenomelia also had trisomy 18. The distribution of these recognizable phenotypes by type of neural tube defect is shown as **Table IV**.

Overall, 139 of 300 (46.3%) neural tube defects were accompanied by other anomalies that did not comprise clinically recognizable phenotypes (Category III, **Table VI**). Among these, the most common anomalies associated with spina bifida were cardiac and genitourinary defects, with anencephaly were cleft lip/palate and cardiac defects, and with encephalocele were cardiac defects and cleft lip/palate.

In this 26-year study, the presence or absence of maternal diabetes was known for 1095 of the 1301 pregnancies (**Table VII**; available at www.jpeds.com). Pregestational diabetes existed in 42 pregnancies that resulted in isolated neural tube defects (12 spina bifida, 21 anencephaly, 9 encephalocele) and in 8 pregnancies that resulted in neural tube defects with other anomalies (4 spina bifida, 1 anencephaly, 3 encephalocele). Gestational diabetes



Project Year	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Spina Bifida	0.97	0.78	0.62	0.47	0.61	0.37	0.31	0.44	0.43	0.38	0.38	0.33	0.40	0.34	0.43	0.30	0.40	0.32	0.41	0.35	0.31	0.36	0.56	0.38	0.21	0.32
Isolated	0.85	0.70	0.58	0.43	0.53	0.33	0.29	0.36	0.28	0.27	0.23	0.25	0.31	0.31	0.30	0.28	0.28	0.22	0.31	0.28	0.24	0.29	0.31	0.26	0.09	0.18
With Anomalies	0.12	0.08	0.04	0.04	0.08	0.04	0.02	0.09	0.14	0.11	0.14	0.09	0.09	0.03	0.13	0.02	0.13	0.10	0.10	0.07	0.07	0.07	0.26	0.12	0.12	0.14

Project Year	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Ancencephaly	0.67	0.57	0.41	0.56	0.59	0.39	0.40	0.43	0.39	0.40	0.25	0.39	0.24	0.34	0.27	0.53	0.26	0.34	0.19	0.28	0.33	0.31	0.26	0.22	0.24	0.19
Isolated	0.42	0.51	0.33	0.48	0.57	0.24	0.27	0.41	0.30	0.24	0.20	0.32	0.24	0.29	0.24	0.42	0.24	0.22	0.14	0.28	0.30	0.26	0.21	0.16	0.19	0.18
With Anomalies	0.24	0.06	0.08	0.08	0.02	0.15	0.13	0.02	0.09	0.16	0.05	0.07	0.00	0.05	0.03	0.11	0.02	0.12	0.05	0.00	0.03	0.05	0.05	0.07	0.05	0.02

Project Year	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Encephalocele	0.24	0.17	0.25	0.21	0.13	0.20	0.16	0.09	0.12	0.14	0.13	0.09	0.12	0.13	0.16	0.16	0.15	0.07	0.17	0.03	0.09	0.17	0.15	0.19	0.09	0.11
Isolated	0.18	0.06	0.17	0.14	0.10	0.13	0.15	0.07	0.09	0.05	0.05	0.05	0.09	0.08	0.11	0.09	0.06	0.05	0.09	0.03	0.03	0.09	0.07	0.10	0.05	0.00
With Anomalies	0.06	0.11	0.08	0.08	0.04	0.07	0.02	0.02	0.04	0.09	0.07	0.04	0.03	0.05	0.05	0.06	0.08	0.02	0.09	0.00	0.05	0.09	0.09	0.09	0.03	0.11

Figure 1. Continued.

occurred in 41 pregnancies that resulted in isolated neural tube defects (19 spina bifida, 13 anencephaly, 9 encephalocele) and in 16 pregnancies that resulted in neural tube defects with other anomalies (6 spina bifida, 4 anencephaly, 6 encephalocele). Valproate was taken in 7 pregnancies that resulted in isolated spina bifida and in 4 pregnancies that resulted in neural tube defects with other anomalies. Obesity as an independent risk factor was identified in 243 (25.6%) of 916 mothers on whom height and weight were known (**Table VII**).

The 62 affected twins and multiple births included 3 conjoined twins, all 3 affected with spina bifida and other anomalies (1 with diaphragmatic hernia, 1 with imperforate anus, and 1 with cleft lip/palate and ambiguous genitalia). In 49 of the sets of twins, only 1 of the twins was affected (25 with spina bifida, 18 with anencephaly, and 6 with encephalocele). In 3 instances both twins had an neural tube defect. One set had anencephaly in one twin and encephalocele in the other. In 2 sets, both twins had spina bifida. Three sets of triplets occurred and one triplet in each set had spina bifida. One infant in a quadruplet pregnancy had anencephaly. Twelve of the affected twins or multiple births occurred in the prefortification years and 50 in the fortification years (**Table VIII**; available at www.jpeds.com).

In a subset of 707 neural tube defects (695 occurrent, 12 recurrent) who enrolled in the South Carolina Neural Tube

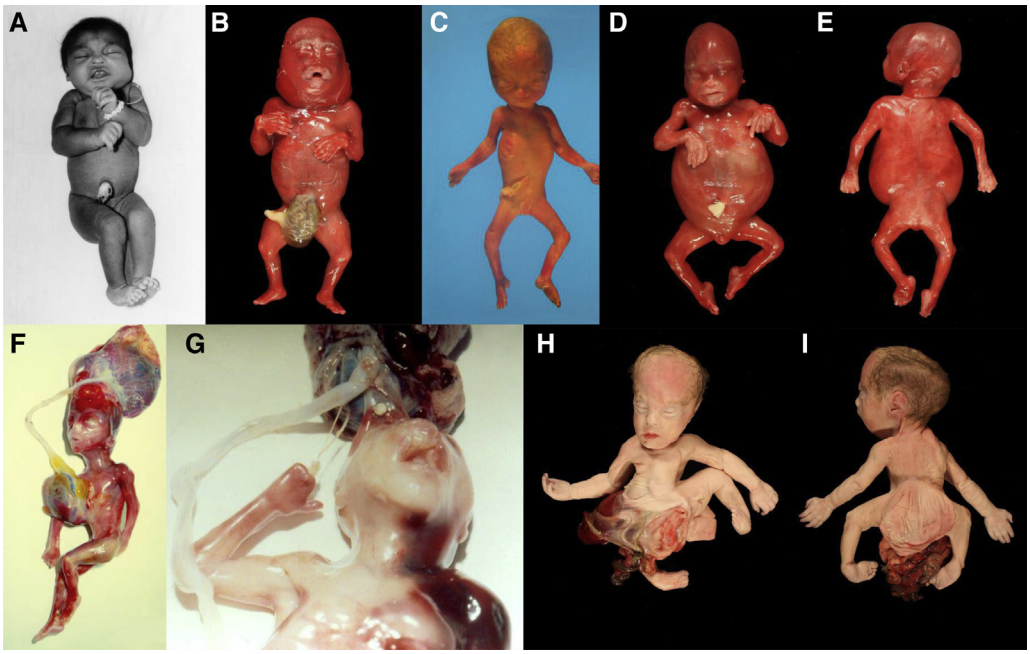


Figure 2. Findings in the 3 most common chromosome aberrations associated with **A-C**, neural tube defects **D, E,** in Meckel syndrome, **F, G,** in amniotic band syndrome and **H, I,** in OEIS syndrome. **A,** Trisomy 18 with microcephaly, overlapping fingers, and rocker bottom feet. **B,** Trisomy 13 with microcephaly, cleft lip/palate, and polydactyly. **C,** Triploidy with craniosomatic disproportion and syndactyly. **D** and **E,** Meckel syndrome with small occipital encephalocele, enlarged abdomen due to cystic kidneys, and polydactyly. **F,** Amniotic band syndrome with cranio-placental fusion and digit amputations. **G,** Amniotic band syndrome with facial clefting, syndactyly, digit amputations, and cranial disruption. **H** and **I,** OEIS syndrome.

Defects Prevention Program, more complete family history, maternal health information, pregnancy history, folic acid use, dietary recall, and demographic data were collected. Folic acid supplements in doses of 400-4000 μg/d were used in the periconceptional period of 192 occurrent pregnancies (35 in the prefortification years, 157 in the fortification years), 142 (74%) of which resulted in isolated neural tube defects and 50 (26%) of which resulted in nonisolated neural tube defects (**Table IX**; available at www.jpeds.com). In total, 400 μg was used in 88 of these pregnancies, 800 μg in 64 pregnancies, 1000 μg in 28 pregnancies, 1200-2200 μg in 11 pregnancies, and 4000 μg in 1 pregnancy.

In addition, 311 women with previous neural tube defect pregnancies took 4000 μg/d folic acid in a subsequent pregnancy. Only one isolated neural tube defect and three non-isolated neural tube defects occurred among these pregnancies.

Prefortification Years

During the 6 years of the prefortification era (1992-1998), 363 neural tube defects were identified in South Carolina; 84% (305/363) were isolated and 16% (58/363) had additional major malformations (**Table II**). This represents a significantly greater percentage of isolated cases (OR 1.828, 95% CI 1.350-2.525, $P < .001$) when compared with the percentage in the fortification years. Encephalocele had the lowest percentage of isolated cases (35/55, 63.6%) and spina bifida had the greatest percentage of isolated cases (149/165, 90.3%). Anencephaly was accompanied by other anomalies in 15.4% of occurrences (22/143).

The 58 nonisolated neural tube defects included 6 (10%) with a chromosomal etiology, 7 (12%) with Meckel syndrome, 14 (24%) with amniotic bands or limb–body wall defect, 1 with short cord syndrome, and 30 (55%) with defects that did not comprise recognizable phenotypes (**Tables IV**, **V**, and **VI**).

Fortification Years

During the 20 years of the fortification era (1999-2018), 938 neural tube defects were identified in South Carolina: 696 (74.2%) were isolated and 242 (25.8%) had other major malformations. Among the nonisolated neural tube defects, a chromosomal aberration, a single-gene disorder, a teratogenic exposure, or a clinically recognizable pattern of malformations (Categories Ia, Ib, and II) were identified in 161 of 1001 (16%). A chromosomal etiology was most common (71/242, 29.3%). Single-gene etiologies included Meckel syndrome (n = 11) and a single *CASP9* mutation. The recognizable phenotypes of uncertain etiology (Category II) included amniotic bands (n = 31), limb–body wall defect (n = 3), OEIS complex (n = 12), short cord syndrome (n = 2), hemifacial microsomia (n = 3), sirenomelia (n = 3), and renal and Müllerian dysgenesis (n = 3).

Among the nonisolated neural tube defects that did not comprise a recognizable phenotype (Category III), cardiac malformation was the most common associated defect

Table V. Chromosomal aberrations including microdeletions and microduplications (n = 77) among 300 nonisolated neural tube defects (prefortification years, fortification years)

Aberrations	SB*	A†	E‡	Total
Trisomy 18	32 (1, 31)	4 (0, 4)	4 (1, 3)	40 (2, 38)
Triploidy	8 (0, 8)	0	0	8 (0, 8)
Trisomy 13	5 (1, 4)	0	3 (0, 3)	8 (1, 7)
Trisomy 21	1 (0, 1)	0	0	1 (0, 1)
Mosaic trisomy 22	0	0	1 (1, 0)	1 (1, 0)
XXX	1 (0, 1)	0	0	1 (0, 1)
XXY	0	1 (0, 1)	0	1 (0, 1)
Mosaic trisomy 9	1 (0, 1)	0	1 (0, 1)	2 (0, 2)
Mosaic trisomy 8	1 (0, 1)	0	0	1 (0, 1)
Deletion 1p	0	0	1 (1, 0)	1 (1, 0)
Deletion 13q	0	1 (0, 1)	2 (0, 2)	3 (0, 3)
Ring 13	0	1 (1, 0)	0	1 (1, 0)
Deletion 15q	1 (0, 1)	0	1 (0, 1)	2 (0, 2)
Deletion 16p	1 (0, 1)	0	0	1 (0, 1)
Deletion 18p	0	1 (0, 1)	0	1 (0, 1)
Deletion 21q	1 (0, 1)	0	0	1 (0, 1)
Translocation 15/20	0	0	1 (0, 1)	1 (0, 1)
Marker	1 (0, 1)	0	0	1 (0, 1)
Tetrasomy 1q	0	1 (0, 1)	0	1 (0, 1)
Duplication 2p	0	1 (0, 1)	0	1 (0, 1)
	53 (2, 51)	10 (1, 9)	14 (3, 11)	77 (6, 71)

*Includes 1 case with multiple vertebral anomalies but no external spina bifida.
†Includes 4 cases of craniorachischisis and 8 cases of iniencephaly.
‡Includes 2 cases of cutis aplasia but no external encephalocele.

(43/139, 31%) and facial clefting was second most common (30/139, 22%) (Table VI).

Discussion

From the landmark studies of Smithells et al, the United Kingdom Medical Research Council, and Czeizel et al in the 1980s and early 1990s, it became clear that enhanced folic acid intake in the periconceptional period would lower the occurrence and recurrence rates of neural tube defects.[10-12] Although it might be assumed that the folate-resistant neural tube defects would be predominantly those caused by chromosomal aberrations, single-gene disorders, and environmental influences unrelated to folate metabolism, this appears to not be the case. Although there was a significantly greater percentage of syndromal neural tube defects in the fortification years, the majority of folate-resistant neural tube defects continued to be isolated malformations.

Neural tube defects are the most common of serious malformations affecting the brain and spine. Neural tube defect prevalence varies widely among different populations, with a very high prevalence recorded in some locations (eg, >10 per 1000 live births and fetal deaths in Ukraine, China, and Ethiopia) and very low prevalence in others (eg, <1 per 1000 live births and fetal deaths in the US and Canada).[6,13-17] The latest population-based prevalence of neural tube defects in the US is for the years 2010-2014.[17] The combined prevalence for the 3 types of neural tube defects was 0.735 in 1000 live births and fetal deaths, which is nearly equivalent to the prevalence in South Carolina (0.747/1000 live births and fetal deaths) for those years and greater than the prevalence in South Carolina for year 26 (0.56/1000 livebirths and fetal deaths) of the survey presented here. The national figures reported by Mai et al do not separate isolated and nonisolated neural tube defects.[17]

Table VI. Nonisolated neural tube defects (n = 139) that did not comprise recognizable phenotypes–Category III (prefortification years, fortification years)

Type of neural tube defects	Holopro-sencephaly	Ocular anomaly	CL/CP	Cardiac	Diaphragmatic hernia	GI	GU	Limb	Other
Spina bifida	4 (3, 1)	2 (0, 2)	5 (0, 5)	18 (1, 17)	4 (3, 1)	15 (2, 13)	17 (4, 13)	8 (0, 8)	4* (0, 4)
Anencephaly	2† (0, 2)	2 (0, 2)	20 (7, 13)	14 (4, 10)	5 (3, 2)	5 (0, 5)	4 (2, 2)	10 (2, 8)	2‡ (0, 2)
Encephalocele	3 (0, 3)	5 (1, 4)	13 (1, 12)	18 (2, 16)	1 (1, 0)	4 (0, 4)	7 (0, 7)	0	1§ (0, 1)
All neural tube defects	9 (3, 6)	9 (1, 8)	38 (8, 30)	50 (7, 43)	10 (7, 3)	24 (2, 22)	28 (6, 22)	18 (2, 16)	7 (0, 7)

CL/CP, cleft lip/cleft palate; *GI*, gastrointestinal abnormalities; *GU*, genitourinary abnormalities.
Note that some cases had more than 1 other anomaly.
*Includes 1 laryngeal atresia, 1 cystic lungs, 1 laryngeal paralysis, and 1 multiple anomalies not specified.
†Diagnosed from facial manifestations.
‡Includes 1 microtia and 1 multiple anomalies not specified.
§Includes 1 craniosynostosis.

Most neural tube defects are sporadic, and specific genetic or environmental causes have been identified in <20% of cases. As in the survey reported here, numerical chromosomal aberrations are found in ~5% of cases, genomic microdeletions or microduplications in <1%, and single-gene disorders in <2%.[15-23] Environmental causes include pregestational maternal diabetes (~5%), maternal obesity (~10%), maternal hyperthermia (<1%), prenatal exposure to anticonvulsants (<1%), and prenatal exposure to other medications, pollutants, and toxins (unknown).[24-26] In South Carolina, the percentage of women with periconceptional obesity (body mass index >29 kg/m^2) during the period 1992-2018 was 25.6%, more than twice the percentage reported by Shaw et al in women from California.[25] The recurrence risk for neural tube defects of 3%-5% is consistent with multifactorial causation with contributions from a large number of genetic and environmental influences. Although more than 250 different genes are involved in neural tube closure in mouse, only a few of them, notably genes involved in planar cell polarity and folate metabolism, have been implicated in human neural tube defects.[27-29]

Chromosomal aneuploidies, segmental deletions, and segmental duplications figure prominently in the causation of neural tube defects.[5] In this survey, chromosomal aberrations, including microduplications and microdeletions, accounted for 5.9% of all neural tube defects and 25.7% of 300 neural tube defects with other major malformations (**Table V**). In the prefortification years 6 of 58 (10.3%) of neural tube defects with other anomalies had chromosomal aberrations. In the fortification years 71 of 242 (29.3%) of neural tube defects with other anomalies had chromosomal aberrations. Spina bifida had the greatest rate of chromosomal errors (53/594, 8.9%), encephalocele the second greatest (14/202, 6.9%), and anencephaly the lowest (10/505, 2.0%).

Meckel syndrome, an autosomal-recessive disorder, is recognized as the most common single-gene condition among neural tube defects (**Figure 2** and **Table IV**). In addition to an encephalocele or other central nervous system anomaly, Meckel syndrome typically has a triad of other findings: polydactyly, cystic kidneys, and hepatic fibrosis. In this survey, 18 cases of Meckel syndrome were identified, comprising 6% of neural tube defects with associated anomalies and 1.4% of all neural tube defects. The only other single-gene disorders were biallelic mutations in *APAF1* (1 case) and *CASP9* (1 case).[30]

Two recognizable phenotypes of unknown cause (Category II)—OEIS complex and amniotic bands/limb–body wall defect—number prominently in this survey and others.[4,5] Both occur as sporadic conditions with amniotic bands/limb–body wall defect being the more common and more variable in craniofacial, central nervous system, ventral body wall, and limb manifestations (**Figure 2**). The neural tube defect most commonly affects the cranium but may be difficult to classify. When the cranium is completely or partially absent, the defect may be considered anencephaly or meroanencephaly. When central nervous system tissues extend beyond the cranial vault but remain skin covered, the defect may be considered to be single or multiple encephaloceles. Atypical facial clefts that do not follow the typical lines of clefts often accompany the cranial malformation. Limb defects are also variable and include single-limb amelia, distal limb or digit amputations, syndactyly, and ring constrictions. Strands of amnion may be found attached to any of the craniofacial, truncal, or limb defects and/or floating free in the amniotic fluid cavity. There is no consensus regarding the origin of amniotic bands or whether the associated neural tube defects are primary or secondary.[31-33]

Because of the overlapping findings, amniotic bands syndrome and limb–body wall defect are classified together in this survey. The 43 cases comprised 3.3% of all neural tube defects and 14.3% of neural tube defects with other anomalies. Amniotic bands/limb–body wall defect accounted for 2.5% of all neural tube defects in the prefortification years and 3.6% of all neural tube defects in the fortification years. OEIS complex was diagnosed in 12 cases comprising 0.9% of all neural tube defects and 4% of neural tube defects with other anomalies (**Table IV**).

Maternal diabetes, obesity, and valproic acid exposure are the best known of a limited number of environmental influences which increase the risk of neural tube defects with or without associated anomalies.[25,34-37] Warfarin exposure and maternal hyperthermia have been associated with neural tube defects and other anomalies.[38-40] Anencephaly and spina bifida have been noted in infants with prenatal exposure to the folic acid antagonist, aminopterin, and prenatal exposure to methimazole, an antithyroid medication, has been associated with cranial aplasia cutis, choanal atresia and other anomalies.[41,42]

The continued occurrence of neural tube defects, the majority of which are isolated, after folic acid fortification of cereal grain flours suggests that additional prevention measures are necessary to further reduce the prevalence of these serious defects of the brain and spine. Measures that potentially could contribute to further reduction include identification of cryptic environmental or genetic/genomic influences that could be mitigated preconceptionally, daily intake of a greater dose of folic acid for all women or for women in the childbearing years, or specifically for women with obesity, diabetes, or epilepsy treated with valproic acid or carbamazepine, and the addition of other micronutrients, such as vitamin B12 or other one-carbon sources, to the prevention strategy.[16,43,44] Failure to consider additional measures is equivalent to stating that the continued occurrence of 35 isolated neural tube defects per year in South Carolina and more than 2500 isolated neural tube defects per year in the US is acceptable.

Lowry et al report that more than 20% of women of reproductive age did not achieve the optimal red blood cell folate concentration for neural tube defect prevention (906 nmol/L) after folic acid fortification of cereal grain flours in

Canada.[16] Fayyaz et al[45] found similar suboptimal red blood cell folate levels during the first 2 trimesters of pregnancy, but noted that deficiencies of vitamins B12 and B6 were rare (<1%).

Although 4000 μg/d folic acid was used in the Medical Research Council's neural tube defect recurrence study without reported adverse effects and is currently the Center of Disease Control and Prevention's recommendation for recurrence prevention, increasing the daily dose of folic acid for all women of childbearing age must be weighed against potential side effects. Patel and Sobczyńska–Malefora have enumerated the potential effects that might occur in certain populations, including diarrhea, rashes, sleep disturbances, masking of vitamin B12 deficiency–related neurologic damage, insulin resistance, increase in cancer risk or promotion of the growth of pre-existing cancers, and lowering the effectiveness of anticonvulsant medications.[46] Other concerns relate to potential effects on the fetus, the most plausible being alteration of the methylation profile. In contrast, Wald et al have suggested that the risks are overstated and that the concept of a tolerable upper intake level of folic acid should be abandoned.[47]

A strength of the study is the complete or nearly complete ascertainment and classification of neural tube defects that occurred before and after fortification over 26 years in a state with a historically high neural tube defect occurrence rate. No claim for complete ascertainment of all neural tube defects can be made because early pregnancy spontaneous and induced terminations and out-of-state terminations could have eluded the surveillance system. Other limitations include the inability to examine and to conduct comprehensive laboratory testing on all neural tube defect fetuses/infants, the inability to obtain serum and erythrocyte folate levels on the mothers, and incomplete information on periconceptional folic acid us especially during the prefortification years. Hispanic families typically use unfortified corn flour, so the assumption that they received adequate folic acid from their diet during the fortification era is incorrect. Eight percent of neural tube defects in the 26-year study period were born into Hispanic families. Although the impact of early pregnancy terminations on the results could not be determined, it is plausible that nonisolated neural tube defects might lead to spontaneous or medical terminations more frequently than isolated neural tube defects. Further, it would have been optimal to have observations during a greater number of prefortification years.

Reduction in the occurrence and recurrence of neural tube defects with folic acid may be properly considered the birth defects prevention success story of the past several decades. This reduction, achieved by folic acid supplementation in the periconceptional period and by folic acid fortification of cereal grain flours, has been most notable in locations with the greatest rates of neural tube defects. Some authorities consider that an irreducible rate of neural tube defects is approaching or has been reached in areas with folate-fortified cereal grain products. Yet, neural tube defects continue to occur and the majority of these are isolated defects, presumably of the type most amenable to folate prevention. ■

We thank the many families and clinicians who have contributed information about neural tube defects that have occurred in South Carolina and to the Birth Defects Program of the SC Department of Health and Environmental Control through which surveillance for neural tube defects is conducted.

Submitted for publication Dec 13, 2019; last revision received Jun 15, 2020; accepted Jul 1, 2020.

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Data Statement

Data sharing statement available at www.jpeds.com.

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Table I. Outcome of pregnancies with neural tube defects in 6 prefortification and 20 fortification years

	Year						
Prefortification	**1**	**2**	**3**	**4**	**5**	**6**	**Total**
Live births	12	34	27	23	30	25	151
Terminations	12	40	36	38	34	20	180
Fetal demise	4	1	1	2	1	0	9
Stillbirths	2	3	1	0	4	6	16
Spontaneous abortion	1	2	1	1	1	1	7
Unknown	0	0	0	0	0	0	0
Total neural tube defects	31	80	66	64	70	52	363
Pregnancies	21 512	65 430	63 063	62 736	63 564	63 965	340 270
Livebirths	16 382	52 359	51 162	51 057	51 930	53 426	276 316
Fetal deaths	181	568	513	515	547	523	2847
Terminations	4949	12 503	11 388	11 164	11 087	10 016	61 107

	Year																				
Fortification	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**Total**
Live births	18	26	17	20	19	20	23	24	37	25	25	19	25	18	23	25	37	32	18	24	475
Terminations	20	18	26	24	16	18	16	23	11	32	18	18	15	15	12	6	7	0	2	2	299
Fetal demise	0	0	1	2	0	0	0	1	0	0	0	0	0	1	1	16	13	9	10	6	60
Stillbirths	10	9	7	3	5	7	5	2	5	5	6	6	5	4	6	2	0	5	1	0	93
Spontaneous abortion	0	1	2	2	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	8
Unknown	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3
Total neural tube defects	48	54	53	51	42	46	44	50	54	63	50	43	45	38	42	49	57	46	31	32	938
Pregnancies	65 251	66 278	66 445	66 050	67 125	67 560	69 379	74 437	76 244	74 989	73 315	70 960	68 553	67 510	66 499	66 925	69 455	68 939	68 184	67 830	1381 921
Live births	54 488	55 650	55 802	54 777	55 209	56 273	57 289	61 028	62 748	63 041	61 281	58 914	57 585	57 160	56 832	57 409	58 009	57 537	57 107	56 759	1154 894
Fetal deaths	523	561	563	532	534	505	517	594	589	551	563	551	520	515	460	484	455	454	411	360	10 240
Terminations	10 240	10 067	10 080	10 741	11 382	10 782	11 573	12 815	12 907	11 397	11 471	11 495	10 448	9835	9207	9032	10 991	10 948	10 666	10 711	216 787

Table II. Isolated and nonisolated neural tube defects in all surveillance years, South Carolina

Type of neural tube defects	All 26 surveillance years	Prefortification years (October 1, 1992-September 30, 1998)	Fortification years (October 1, 1998-September 30, 2018)	Statistical significance
All neural tube defects	1301	363	938	
Isolated	1001 (77%)	305 (84%)	696 (74%)	OR 1.828, 95% CI 1.330-2.525, $P \leq .0001$*
Nonisolated	300 (23%)	58 (16%)	242 (26%)	OR 0.5469, 95% CI 0.3960-0.7521, $P \leq .0001$*
Spina bifida	594	165	429	
Isolated	466 (78%)	149 (90%)	317 (74%)	OR 3.290, 95% CI 1.886-5.676, $P \leq .0001$*
Nonisolated	128 (22%)	16 (10%)	112 (26%)	OR 0.3039, 95% CI 0.1762-0.5303, $P \leq .0001$*
Anencephaly	505	143	362	
Isolated	416 (82%)	121 (85%)	295 (81.5%)	OR 1.249, 95% CI 0.7354-2.105, ns†
Nonisolated	89 (18%)	22 (15%)	67 (18.5%)	OR 0.8005, 95% CI 0.4751-1.360, ns†
Encephalocele	202	55	147	
Isolated	119 (59%)	35 (64%)	84 (57%)	OR 1.313, 95% CI 0.7077-2.435, ns†
Nonisolated	83 (41%)	20 (36%)	63 (43%)	OR 0.7619, 95% CI 0.4106-1.413, ns†

ns, not significant.
*Significantly greater percentage of isolated defects and significantly smaller percentage of nonisolated defects in prefortification years compared with fortification years ($P < .0001$).
†Greater percentage of isolated defects and smaller percentage of nonisolated defects in prefortification years ns.

Table III. Prevalence of neural tube defects in the prefortification and fortification years by race

Race	Prevalence (neural tube defects/deliveries)	
	Prefortification years (1992-1998)	Fortification years (1999-2018)
White	265/175 284 (1/661) [A: 105, SB: 124, E: 36]	562/684 464 (1/1218) [A: 228, SB: 249, E: 85]
Black	86/97 835 (1/1138) [A: 32, SB: 35, E: 19]	250/380 749 (1/1523) [A: 85, SB: 125, E: E−40]
Hispanic	7/4833 (1/690) [A: 3, SB: 4, E: E−0]	100/90 193 (1/902) [A: 41, SB: 41, E: 18]
Other/unknown	5/3847 (1/769) [A: 2, SB: 3, E: 0]	26/27 032 (1/1040) [A: 8, SB: 14, E: 4]
All races	363/281 799 (1/776) [A: 142, SB: 166, E: 55]	938/1 182 438 (1/1261) [A: 362, SB: 429, E: 147]

A, anencephaly; *E*, encephalocele; *SB*, spina bifida.

Table IV. Type of neural tube defects associated with chromosomal aberrations, Meckel syndrome, and selected other malformations

Associated defects	SB	A	E
Chromosome (n = 77*)	53 (69%)	10 (13%)	14 (18%)
Amniotic bands/L-BWD (n = 43)	5 (12%)	21 (49%)	17 (40%)
Diaphragmatic hernia (n = 16†)	8 (50%)	5 (31%)	3 (18%)
Meckel (n = 18)	0	1 (6%)	17 (94%)
Holoprosencephaly (n = 15‡)	6 (40%)	2 (13%)	7 (47%)
OEIS (n = 12)	12 (100%)	0	0

L-BWD, limb–body wall defect.
*Among the chromosome aberrations were 2 copy number variations <500 kb (1 spina bifida with 435 kb loss of 16p and 1 anencephaly with 334 kb loss of 18p).
†Six neural tube defects with diaphragmatic hernia had chromosome abnormalities (2 trisomy 18, 2 trisomy 13, 1 mosaic trisomy 9, 1 XXY).
‡Six neural tube defects with holoprosencephaly had a chromosome or single gene abnormality (3 trisomy 13, 2 13q deletion, 1 Meckel).

Table VII. Maternal diabetes mellitus and obesity as risk factors

	Prefortification			Fortification years		
Risk factors	All neural tube defects	Isolated	Nonisolated	All neural tube defects	Isolated	Nonisolated
Gestational diabetes	19	15	4	38	26	12
Spina bifida	9	9	0	16	10	6
Anencephaly	6	4	2	11	9	2
Encephalocele	4	2	2	11	7	4
Pregestational diabetes	10	9	1	40	33	7
Spina bifida	3	3	0	13	9	4
Anencephaly	5	4	1	17	17	0
Encephalocele	2	2	0	10	7	3
Without diabetes	270	226	44	718	520	198
Unknown	64	56	8	142	117	25
Total neural tube defects	363	306	57	938	696	242
All mothers with BMIs available	241	204	37	675	493	182
BMIs >29 kg/m^2 as an independent risk factor	59	56	3	184	147	37

BMI, body mass index.

Table VIII. Twins and other multiple births with neural tube defects (n = 62)

Types of multiple birth	Prefortification years (6)	Fortification years (20)	All years (26)
Twins—1 affected	8	41	49
Spina bifida	5	20	25
Anencephaly	3	15	18
Encephalocele	0	6	6
Twins—both affected	1	2	3
Spina bifida	2	2	4
Anencephaly	0	1	1
Encephalocele	0	1	1
Conjoined twins	1	2	3
Spina bifida	1	2	3
Anencephaly	0	0	0
Encephalocele	0	0	0
Quads, 1 w/anencephaly	1	0	1
Triplets, 1 w/spina bifida	0	3	3

Table IX. Use of folic acid supplements in a subset of 707 neural tube defect pregnancies following which the mothers enrolled in the recurrence prevention component of the South Carolina Neural Tube Defects Prevention Program

Folic acid dose/d	Prefortification years	Fortification years	All years
Occurrent neural tube defects	252 (198 isolated)	443 (328 isolated)	695 (526—76% isolated)
Folic acid used	35 (30 isolated)	157 (112 isolated)	192 (142—74% isolated)
400-1000 μg	33 (28 isolated)	147 (106 isolated)	180 (134—74% isolated)
1200-2200 μg	2 (2 isolated)	9 (6 isolated)	11 (8—73% isolated)
4000 μg	0	1 (0 isolated)	1 (0 isolated)
Recurrent neural tube defects	3 (2 isolated)	9 (4 isolated)	12 (6—50% isolated)
Folic acid used	0	5 (1 isolated)	5 (1—20% isolated)
400-1000 μg	0	2 (0 isolated)	2 (0 isolated)
1200-2200 μg	0	0	
4000 μg	0	3 (1 isolated)	3 (1—33% isolated)