HHS Public Access

Author manuscript

Birth Defects Res. Author manuscript; available in PMC 2024 January 01.

Published in final edited form as:

Birth Defects Res. 2023 January 01; 115(1): 43–55. doi:10.1002/bdr2.2003.

Patterns of multiple congenital anomalies in the National Birth Defect Prevention Study: Challenges and insights

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Abstract

Background: About 20%–30% of children with birth defects have multiple major birth defects in more than one organ system, often referred to as multiple congenital anomalies (MCAs). Evaluating the patterns of MCAs can provide clues to the underlying causes, pathogenic mechanisms, and developmental pathways. We sought to explore selected patterns of MCAs within the National Birth Defects Prevention Study (NBDPS), a population-based, case–control study that excluded cases attributed to known chromosomal or single-gene abnormalities.

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AUTHOR CONTRIBUTIONS

Meredith M. Howley, A. J. Agopian, Angela E. Lin, Lorenzo D. Botto, Paul A. Romitti, and Marilyn L. Browne conceived the project and designed the analysis. **Eva Williford** conducted the data analysis. **Meredith M. Howley** replicated the analysis. **Angela E. Lin, Lorenzo D. Botto, and Christopher M. Cunniff** conducted the clinical review of case children and provided clinical interpretations. **Meredith M. Howley** drafted the initial manuscript. All authors have critically reviewed, edited, and approved the final manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

Methods: We defined MCAs as having two or more NBDPS-eligible birth defects and calculated the adjusted observed-to-expected ratio for all observed MCA patterns using co-occurring defect analysis.

Results: Of the 50,186 case infants eligible for NBDPS, 2,734 (3.7%) had at least two eligible birth defects. We observed 209 distinct 2-way combinations of birth defects, 297 distinct 3-way combinations, 179 distinct 4-way combinations, and 69 distinct 5-way combinations. Sacral agenesis had the largest proportion of cases with MCAs (70%), whereas gastroschisis had the lowest (3%). Among the cases with MCAs, 63% had a heart defect, 23% had an oral cleft, and 21% had anorectal atresia/stenosis. Of the patterns with adjusted observed-to-expected ratios in the top 20%, most were consistent with the known associations or syndromes, including VATER/VACTERL association and CHARGE syndrome.

Conclusions: Most but not all patterns that had the highest adjusted observed-to-expected ratios were instances of known syndromes or associations. These findings highlight the importance of considering birth defect combinations that suggest syndromic patterns in the absence of a formal syndromic diagnosis. New approaches for screening for sequences and associations, and VATER/VACTERL in particular, in surveillance systems with limited resources for manual review may be valuable for improving surveillance system quality. The observed MCA patterns within NBDPS may help focus future genetic studies by generating case groups of higher yield.

Keywords

co-occurrence; multiple birth defects; multiple congenital anomalies; observed-to-expected ratio

1 | INTRODUCTION

About 20%–30% of children with birth defects have multiple major birth defects in more than one organ system not known to be pathogenetically related, often referred to as multiple congenital anomalies or MCAs (Agopian, Evans, & Lupo, 2018; Calzolari et al., 2014; Garne et al., 2011). Evaluating the patterns of such MCAs can provide clues to the underlying causes, pathogenic mechanisms, and developmental pathways (Friedman, 1992; Kallen & Winberg, 1969). For example, many syndromes (disorders due to a specific factor, typically but not exclusively genetic or chromosomal) are preferentially associated with certain patterns of MCAs. In addition, because several known teratogens, including some infections (e.g., rubella) and medications (e.g., retinoic acid) produce recognizable patterns of MCAs, public health surveillance of cases with MCAs has been proposed as a tool for early detection of new teratogens, which may be missed or delayed by surveillance of isolated birth defects alone (Calzolari et al., 2014; Khoury, Adams, Rhodes, & Erickson, 1987; Khoury et al., 1993, 1994; Mastroiacovo, 1991).

Several challenges make the study of MCAs particularly difficult. MCAs are relatively rare and parsing them into specific combinations of birth defects generates groups that are typically too small for meaningful statistical analysis. Analytically, identifying new MCAs relies not only on statistical analysis but also on clinical evaluation and biological understanding. Several statistical methods for evaluating patterns of MCAs have been proposed and used, but no single approach has been shown to be ideal (Agopian et al.,

2018; Beaty, Yang, Khoury, Harris, & Liang, 1991; Kallen et al., 1999; Khoury et al., 1993; Khoury, James, & Erickson, 1990).

To attempt to address these challenges, we sought to explore the patterns of MCAs within the National Birth Defects Prevention Study (NBDPS), a large, population-based, case–control study of major structural birth defects (Rasmussen et al., 2003; Reefhuis et al., 2015). Its large sample size, extensive clinical information on case children, exclusion of known genetic and chromosomal syndromes, and systematic review of records by a central team of clinical geneticists improves upon some of the limitations of previous studies. By describing the selected patterns of MCAs among those eligible for NBDPS, we hope to generate hypotheses for future research in identifying causes of birth defects.

2 | METHODS

2.1 | Overall structure of the NBDPS

The NBDPS is a large, multisite, population-based, case–control study of major structural birth defects that included pregnancies ending on or after October 1, 1997 and had estimated dates of delivery on or before December 31, 2011 (Reefhuis et al., 2015). Case children were eligible for the NBDPS if they had one or more of 30 different categories of major birth defects and were ascertained through birth defect surveillance programs in 10 states (Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah). Birth defects that were known or strongly suspected to be caused by a single-gene disorder or chromosomal anomaly were excluded from the NBDPS, meaning that eligible birth defects were thought to be non-syndromic at the time of ascertainment. Clinical information abstracted from medical records was reviewed by clinical geneticists who determined eligibility and classified case children as having birth defects that were isolated (within one organ system), multiple (in more than one organ system and thought to be unrelated pathogenetically), or complex (a group of defects believed to be pathogenetically related, but the primary defect was not apparent; Rasmussen et al., 2003).

2.2 | Definitions and classification of MCAs

In the NBDPS, case children with a defect of interest were classified as having multiple defects if there was at least one additional unrelated major birth defect in another organ system, regardless of whether the observed defect was eligible for the NBDPS. For our analysis, we defined MCAs as NBDPS case children (live born, stillborn, or terminated) with two or more eligible NBDPS defects. Thus, some cases classified by a clinical geneticist as having multiple defects had only one NBDPS-eligible defect and were excluded from our analysis. We focused on patterns of NBDPS-eligible defects for two reasons: the birth prevalence of non-eligible defects was unknown as they were not actively ascertained for the study and non-eligible defects may not have been as rigorously reviewed and classified as NBDPS-eligible defects. We included all NBDPS-eligible birth defects in our analysis, combining the defects into 23 categories (Table 1; Botto et al., 2007; Rasmussen et al., 2003; Reefhuis et al., 2015). A child with more than one NBDPS-eligible defect in the same defect category (e.g., microphthalmia with congenital cataract or microphthalmia

on one side and anophthalmia on the contralateral side) was counted only once within that category (e.g., eye defects). A child with multiple major birth defects that are related due to a common pathogenetic mechanism (e.g., spina bifida and talipes equinovarus) was also classified as an isolated case.

NBDPS researchers chose to study certain MCA associations as “entities” rather than separate defects, depending on what was known about the etiologies of these associations at the start of the study. Amniotic band sequence-limb-body wall complex (ABS-LBWC), cloacal exstrophy, and holoprosencephaly were understood to result from an early developmental disturbance. Children diagnosed with ABS-LBWC and another eligible defect that is part of the complex (anencephaly, encephalocele, cleft lip and/or palate, anophthalmia/microphthalmia, limb deficiency, omphalocele, or gastroschisis) were generally counted only as an ABS-LBWC case within NBDPS (if it was thought that the other defect was associated with ABS). Similarly, cloacal exstrophy cases, many of which met the criteria for OEIS complex (Omphalocele, Exstrophy of the cloaca, Imperforate anus, and Spinal defects) were counted only as a cloacal exstrophy case with the other component defects (e.g., bladder exstrophy, anorectal atresia, or omphalocele) not included in their respective case groups. Lastly, children diagnosed with holoprosencephaly who had cleft lip and/or microphthalmia were counted only as a holoprosencephaly case and not counted in the other case groups. Thus, children with either ABS-LBWC, cloacal exstrophy, or holoprosencephaly included in this analysis had one or more additional defects outside those included in their respective complexes.

For VATER/VACTERL association (Vertebral anomalies, Anorectal defects, Cardiac defects, Tracheoesophageal fistula, Esophageal atresia, Renal defects, Limb defects) and CHARGE (Coloboma, Heart defect, Atresia of choanae, Retardation of growth and development, Genital hypoplasia, Ear defect) syndrome, because less was known about the etiologies of these associations, each of the diagnosed NBDPS-eligible defects for a case child was coded so that the component defects were counted separately (Solomon, 2011, 2018). The VATER/VACTERL phenotype did not affect the MCA classification; presence of a birth defect outside the VATER/VACTERL phenotype was not required to meet the definition of MCA. Likewise, cases with CHARGE syndrome were included in NBDPS, and each diagnosed, NBDPS-eligible defect was counted separately (Blake et al., 1998; Pagon, Graham Jr., Zonana, & Yong, 1981). Because of the extensive use of molecular genetic testing to confirm clinically diagnosed cases following the detection of the causative gene *CHD7*, the NBDPS modified inclusion criteria for cases with CHARGE syndrome phenotype with estimated dates of delivery in 2006 or later to exclude those in which a pathogenetic or likely pathogenetic variant of *CHD7* was present (van Ravenswaaij-Arts, Hefner, Blake, & Martin, 2006). Cases with CHARGE syndrome that that had no pathogenic or likely pathogenic variants of *CHD7* or did not have mutation testing performed were included throughout the study. Similar to VATER/VACTERL, NBDPS case children with CHARGE syndrome were classified as having multiple birth defects, regardless of the presence of birth defects outside the CHARGE syndrome phenotype.

2.3 | Statistical analyses of MCAs

For each combination of two or more NBDPS-eligible defects, we estimated the adjusted observed-to-expected ratio (adjusted O:E ratio), using the R-based program Co-Occurring Defect Analysis (CODA) described by Benjamin et al. (Benjamin et al., 2019; Khoury et al., 1990). This method compared the observed prevalence of a particular combination of birth defects to the expected prevalence of the combination of birth defects, based on the prevalence of each birth defect in the combination. We adjusted the O:E ratio for the tendency of birth defects to cluster with major birth defects (Khoury et al., 1990). When calculating the adjusted O:E ratios for MCA patterns involving hypospadias, we only included males. Our analysis focused on the 20% of MCA patterns that had the highest adjusted O:E ratios and were observed in at least five case children (n = 31 patterns).

The NBDPS excluded case children with genetic confirmation of known syndromes. Yet, during the 15-year study period, novel powerful genetic tests were implemented and new genetic syndromes were identified. Thus, we hypothesized that some of the MCA patterns with elevated adjusted O:E ratios may either capture known associations that were included in the NBDPS (e.g., VATER/VACTERL) or syndromes that were unknown or not widely tested for at the time of study inclusion. To investigate this, we reviewed the MCA patterns with the largest adjusted O:E ratios. For the patterns involving more than two birth defects that were not part of the typical VATER/VACTERL phenotype (42 cases representing 7 MCA patterns), clinical geneticists (A.E.L. and C.M.C.) re-reviewed case-level clinical information, and assigned each of the 42 cases into one of four mutually exclusive groups without genetic evidence of the syndrome: definitely syndromic, probably syndromic, possibly syndromic, or not syndromic. We then conducted a sensitivity analysis by removing case children considered “definitely” or “probably” syndromic and re-estimated the adjusted O:E ratios.

2.4 | Additional analyses

We used available information from the state surveillance systems participating in the NBDPS to describe the demographic characteristics of all eligible case children. We used Pearson's chi-square tests to compare the characteristics of case children with MCAs and isolated cases. Demographic information included: child sex, birth outcome (live born/stillborn/termination), plurality (singleton/multiple), maternal age at delivery (<20 years/20–34 years/or 35+ years), maternal race/ethnicity (non-Hispanic White/non-Hispanic Black/Hispanic/other), birth year, and site. For live-born cases, we also compared gestational age at delivery (<37 weeks/37+ weeks) and birth weight (<2,500 g/≥2,500 g). Each NBDPS site and the Centers for Disease Control and Prevention obtained institutional review board approval.

3 | RESULTS

During the study period, 50,186 case children were eligible for the NBDPS (Figure 1). Of these, 7,484 (14.9%) were classified as having multiple major birth defects. We excluded 4,750 case children classified as having multiple defects who had only one NBDPS-eligible defect; Table S1 includes counts of the eligible defects among these excluded cases. In our

analysis, 2,734 case children had at least two NBDPS-eligible defects, 463 had at least 3 NBDPS defects, 95 had at least 4 NBDPS defects, 23 had at least 5 NBDPS defects, 5 had at least 6 NBDPS defects, and 2 had 7 NBDPS defects (Figure 1). The birth defect group with the largest proportion of cases with MCAs in our analysis was sacral agenesis, with 69.5% of sacral agenesis cases having at least 2 NBDPS- defects, whereas only 3.2% of gastroschisis cases had at least 2 NBDPS defects (Table 2). Among the cases with MCAs in our analysis, 63.1% had a CHD, 23.1% had an oral cleft, and 20.8% had anorectal atresia/stenosis.

The 2,734 children with MCAs (defined as two eligible NBDPS defects) comprised 209 distinct 2-way combinations of birth defects. Of these, 86 patterns had 5 or more observed cases and an adjusted O:E ratio greater than 1 (Figure 1). The 463 case children with MCAs with at least 3 NBDPS defects resulted in 297 distinct 3-way MCA patterns, of which 60 patterns had 5 or more observed cases and an adjusted O:E ratio greater than 1. The 95 case children with MCAs with at least 4 NBDPS defects resulted in 179 distinct 4-way MCA patterns, of which 5 patterns had 5 or more observed cases and an adjusted O:E ratio >1. While there were case children with 5, 6, and 7 NBDPS defects, no patterns had 5 or more cases.

The MCA patterns with adjusted O:E ratios in the top 20% are presented in Table 3; Table S2 includes adjusted O: E ratios for all MCA patterns with 5 or more affected cases. The largest adjusted O:E ratio was 106.41 for the 4-way pattern that included anorectal atresia/stenosis, esophageal atresia, limb deficiency, and sacral agenesis, meaning the prevalence of this combination was 106-fold higher than would be expected if the occurrence of these birth defects were independent. Of the 31 MCA patterns with O:E ratios in the top 20%, 21 were comprised of birth defects that seemed consistent with the VATER/VACTERL association. In our sensitivity analysis of 7 MCA patterns with more than 2 birth defects (Table 4), 18 of the 42 cases were thought to be syndromic on re-review. Excluding these individual cases resulted in consistently lower adjusted O:E ratios compared to the main analysis.

Children with MCAs differed from children with isolated defects by several factors (Table 5). Compared to children with isolated defects, cases with MCAs were more frequently stillborn or terminated. Among livebirths, case children with MCAs were more frequently preterm and weighed less than 2,500 g at birth than isolated case children. Compared to isolated case children, case children with MCAs were more frequently from multiple gestations and more frequently reported a race/ethnicity other than White. There were also differences by site and birth year.

4 | DISCUSSION

We report on the MCA patterns among the 2,734 case children within the NBDPS who had at least two NBDPS-eligible defects in different organ systems. As reported in previous studies, children with MCAs were more often stillborn or terminated compared to children with isolated defects, and liveborn children with MCAs were more often born preterm and of low birthweight compared to liveborn children with isolated defects (Calzolari et al., 2014; Toxværd & Garne, 2021). Similar to data from European registries, we did not

find differences by child sex or maternal age at delivery between cases with MCAs and isolated cases (Calzolari et al., 2014). Additionally, we observed significant variations in the prevalence of MCAs by site ranging from 5% to 15%. This may be attributable to differences between sites in factors such as genetic testing, ascertainment of stillbirths and pregnancy terminations (NY and NJ had the lowest percentage of cases with MCAs and only ascertained livebirths in the beginning of the NBDPS), or racial-ethnic distribution (e.g., CA and TX have the highest percentage of cases with MCAs and the highest proportion of Hispanic cases, in whom terminations of pregnancy are less common; Calzolari et al., 2014; de Graaf, Buckley, & Skotko, 2016).

We used the strength of association (measured as adjusted O:E ratios) among individual birth defects to identify and rank specific MCA patterns. Using this approach, we found that most (21 out of the 31) MCA patterns included in the highest 20% of adjusted O:E ratios were those that included typical components of the VATER/VACTERL phenotype. This finding demonstrates that our method of comparing adjusted O:E ratios among cases with MCAs was effective in identifying clinically established birth defect associations and is similar to other studies (Benjamin et al., 2021). Also, most (15 of the 21) MCA patterns in Table 3 that included components of the VATER/VACTERL phenotype included sacral agenesis. Although it is the only eligible vertebral birth defect within NBDPS, sacral agenesis is not a typical component of the standard VATER/VACTERL phenotype (Solomon, 2011). Both sacral agenesis and certain VATER/VACTERL defects (alone or in combination), including anorectal atresia, are commonly reported in diabetic embryopathy. A recent NBDPS analysis reported that while pregestational diabetes was associated with a markedly increased risk for many specific birth defects, the strongest association was observed for sacral agenesis, with an odds ratio of 67.8 (95% confidence interval 37.0, 124.2) (Tinker et al., 2020). Among the interviewed case children with MCAs in our analysis who had sacral agenesis, 23% of mothers reported pregestational diabetes. We speculate that the VATER/VACTERL cases with sacral agenesis may represent a subset with shared etiology or another pattern entirely. Our clinical geneticist review of the two MCA patterns in Table 4 that included anorectal atresia and sacral agenesis as component features showed that the case descriptions indicated either caudal dysplasia, a mixed phenotype (caudal dysplasia and VATER), or an atypical phenotype for caudal dysplasia or VATER/VACTERL. These finding suggest an early insult to the mesoderm may have played a role in the patterns that included anorectal atresia and sacral agenesis.

Additionally, three of the seven MCA patterns in Table 4 were strongly suggestive of CHARGE syndrome (e.g., eye anomaly, congenital heart disease, choanal atresia; van Ravenswaaij-Arts et al., 2006). We observed six case children with the 4-way pattern of CHD, orofacial cleft, limb deficiency, and anotia/microtia (Table 4), of which three were judged to likely be either VATER/VACTERL association or CHARGE syndrome. Interestingly, a possible known association was suggested for only one of the five case children with the final MCA pattern (orofacial cleft, esophageal atresia, and hydrocephaly) listed in Table 4. Thus, most but not all case children with MCA patterns having the highest adjusted O:E ratios may be instances of known syndromes or associations. Those for which known syndromes or associations were not identified may represent syndromes or associations not previously recognized or variations of known patterns. Table S2 contains

all observed MCA patterns involving at least two NBDPS birth defects, which may include unrecognized MCA patterns worth further exploration in other large surveillance data sets.

Although the NBDPS aimed to exclude known syndromes, the ability to do so was dependent on knowledge and genetic testing practices during the study period. The evolution of testing and knowledge over the 15-year study period meant that some unrecognized genetic syndromes may have been inadvertently included, modifying the prevalence and perhaps the distribution of MCAs. Tests such as chromosome microarray, next generation sequencing gene panels, and whole exome sequencing became increasingly utilized diagnostic tests during the study period (Malinowski et al., 2020). The diagnostic yield of approximately 25%–40% for whole exome sequencing alone in the evaluation of fetuses or newborns with MCAs suggests that at least some cases not tested by this modality were actually caused by a genetic abnormality (Fu et al., 2018; Meng et al., 2017). The NBPDS changed eligibility criteria as genetic testing evolved. For example, at the start of NBDPS, cases with CHARGE were considered to be MCAs. Following the discovery of the causative gene, *CHD7*, CHARGE was redefined as a genetic syndrome (OMIM #214800) (Hale, Niederriter, Green, & Martin, 2016; Kancherla et al., 2014; Sanlaville & Verloes, 2007). In our sensitivity analysis, 18 of the 42 case children with MCA patterns with elevated adjusted O:E ratios that included birth defects not part of VATER/VACTERL were thought to be syndromic on re-review. In the current analysis, the inclusion of undiagnosed syndromes may have increased the adjusted O:E ratios for certain MCAs.

Our agnostic approach provides another example of computation-driven techniques for exploring MCAs involving a large number of combinations of birth defects. Although the value of data generated by computation-driven techniques will always be dependent upon the quality of birth defect surveillance data on which the computations are based, such methods can enhance traditional coding and analysis of individual birth defects and could be particularly useful for state and country birth defect surveillance systems lacking manual review for syndromic diagnosis identification. Designating cases as syndromic or as belonging to recognized patterns enables exclusion of those cases from some analyses and a focus on certain groups for other analytic goals. Including these potentially syndromic cases in an analysis may make it difficult to identify other MCAs with a potentially teratogenic cause, as they may have been masked by the stronger syndromic cases. Additionally, it may be that inclusion of undiagnosed genetic conditions attenuated associations of more traditional risk factor-birth defect analyses by including cases for whom environmental exposures would not play a role. The recognition of potential syndromes may in turn help generate future genetic research or improve understating of developmental pathways. The observed MCA patterns may help focus future genetic studies by generating case groups of higher yield for genetic or genomic analyses.

Our analysis was restricted to case children with MCAs defined as having at least two NBDPS-eligible birth defects. Children classified as having multiple birth defects where only one defect was eligible for NBDPS were excluded from our analysis, and these cases account for the majority (63%) of children with multiple birth defects. The prevalence of non-eligible birth defects would be unknown within the NBDPS study population, as only cases with at least one NBDPS defect were included in the study population. Although

this limited our ability to explore all MCA patterns, we were able to leverage NBDPS to explore the co-occurrence of over 20 categories of major structural birth defects. There were differences in the proportion of cases with MCAs across the 10 NBDPS sites, which may suggest differences in genetic testing practices, insurance coverage, or parental consent for testing.

The NBDPS employed strict inclusion criteria and case classification by clinical geneticists, so we expect little misclassification of individual birth defects. By using all eligible birth defect cases, we were able to explore MCA patterns in a large, multisite, population-based sample, which limited the potential for selection bias. The large sample allowed us to assess high order combinations of birth defects and employ an analytic approach that accounted for nonspecific clustering of birth defects.

In combining our agnostic, computation-driven analysis with clinician review of certain MCA patterns, we identified several patterns of birth defects that occurred more frequently than would be expected. Whereas most of these combinations represent clinically recognized MCA associations (e.g., VATER/VACTERL) or syndromes (e.g., CHARGE syndrome), a few others may represent unrecognized recurrent MCA phenotypes that could benefit from further etiologic investigations. For example, we observed five cases with orofacial cleft, esophageal atresia, and hydrocephaly; a nearly 13-fold higher prevalence than would be expected. This combination of birth defects does not fit with known syndromes or associations. Other two-way, three-way, and four-way combinations of birth defects were observed and had more than five affected cases. Although we did not attempt to summarize and interpret these findings, genetic analysis of some of these patterns may uncover unknown genetic causes of birth defects. These findings may be useful to birth defects surveillance programs, which classify cases, especially for surveillance programs without a clinical geneticist reviewer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The authors thank the participating families, scientists, and staff from the NBDPS sites. This project was supported through Centers for Disease Control and Prevention (CDC) cooperative agreements under PA #96043, PA #02081, FOA #DD09-001, FOA #DD13-003, and NOFO #DD18-001 to the Centers for Birth Defects Research and Prevention participating in the National Birth Defects Prevention Study (NBDPS) and/or the Birth Defects Study To Evaluate Pregnancy exposureS (BD-STEPS). The project (A. J. Agopian) was also supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development (R01 HD093660).

DATA AVAILABILITY STATEMENT

The study questionnaires and process for accessing the data used in this study is described at https://www.cdc.gov/ncbddd/birthdefects/nbdps-public-access-procedures.html. The code book may be made available upon request.”

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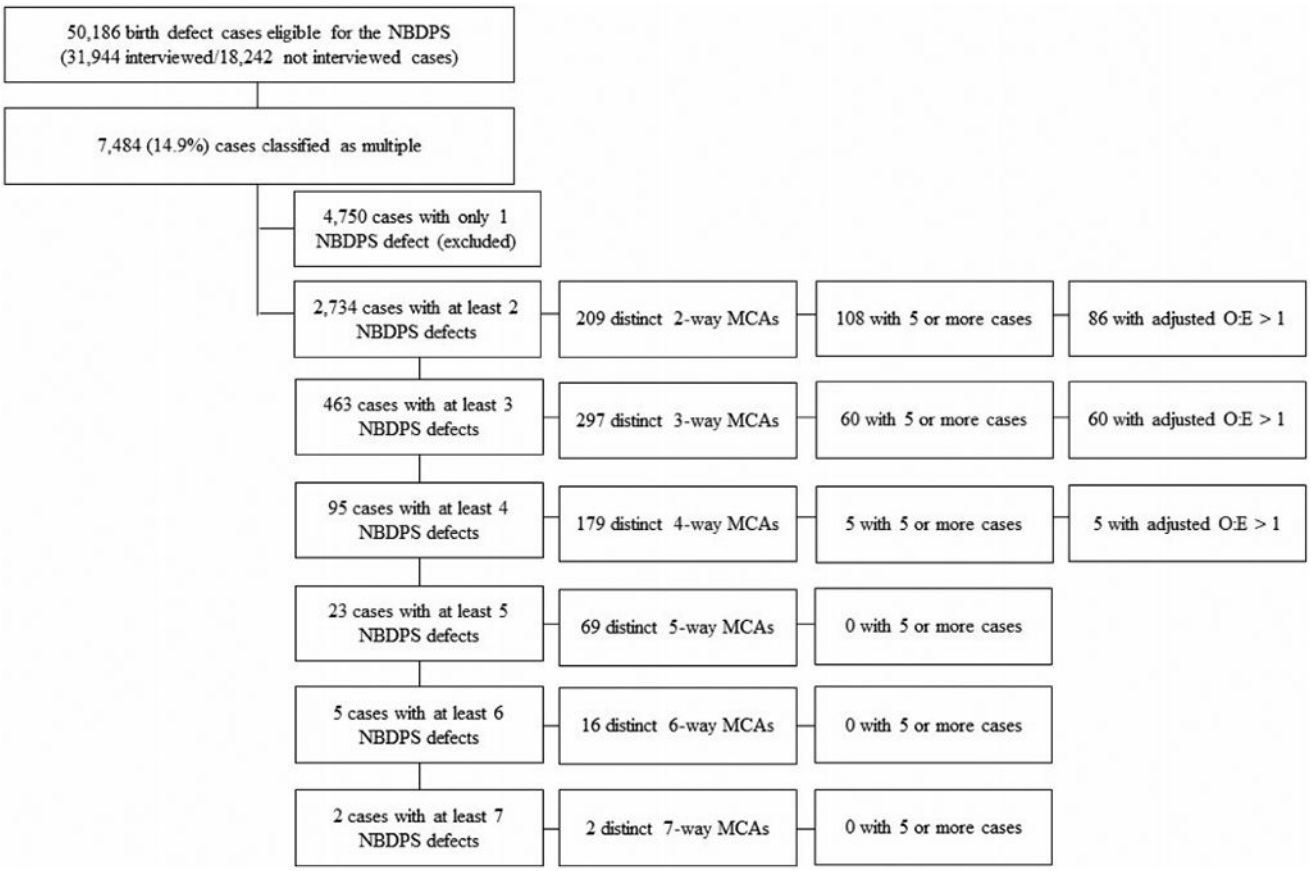

FIGURE 1.

Case children (interviewed and non-interviewed) with multiple eligible birth defects in the National Birth Defects Prevention Study

TABLE 1

Birth defects eligible for the National Birth Defects Prevention Study, by analysis categories

Congenital heart defects (CHD)
Truncus arteriosus
Interrupted aortic arch (types A, B, and not specified)
d-transposition of the great arteries
Tetralogy of Fallot
Double outlet right ventricle
Atrioventricular septal defects
Anomalous pulmonary venous return
Hypoplastic left heart syndrome
Aortic stenosis
Coarctation of the aorta
Pulmonary atresia
Tricuspid atresia
Pulmonary valve stenosis
Ebstein malformation
Ventricular septal defects (VSD)
Atrial septal defects (ASD)
Single ventricle defects
Heterotaxy
Amniotic band syndrome and limb-body wall complex (ABS-LBWC)
Neural tube defects (NTD)
Anencephaly/craniorachischisis
Spina bifida
Encephalocele
Hydrocephaly
Holoprosencephaly
Dandy–Walker malformation or cerebellar hypoplasia (DWM/CH)
Anotia/Microtia
Eye defects
Microphthalmos/anophthalmos
Cataractsa
Glaucoma and other anterior chamber defects
Choanal atresia
Oral clefts
Cleft lip only
Cleft palate only
Cleft lip with cleft palate
Esophageal atresia
Intestinal atresia/stenosis
Small intestinal atresia/stenosis
Duodenal atresia/stenosis
Colonic atresia/stenosis
Anorectal atresia/stenosis
Cloacal exstrophy
Biliary atresia
Hypospadias (second/third degree)
Bilateral renal agenesis/hypoplasia
Bladder exstrophy
Limb deficiency
Longitudinal limb deficiency
Intercalary limb deficiency
Transverse limb deficiency

Limb deficiency, not specified
Craniosynostosis
Diaphragmatic hernia
Omphalocele
Gastroschisis
Sacral agenesis

TABLE 2

Frequency of eligible birth defects among all cases, classified as having multiple defects, and those with at least two eligible birth defects in the National Birth Defects Prevention Study (NBDPS), in descending order of percent multiple congenital anomalies (MCAs) for each birth defect

Birth defects	NBDPS-eligible cases	Cases classified as having multiple defects	Cases with at least two NBDPS-eligible defects		
			Number	Percent	Percent of all MCAs[a]
Sacral agenesis	174	146	121	69.5	4.4
Esophageal atresia	1,071	629	416	38.8	15.2
Cloacal exstrophy	148	59	37	35.0	1.4
Anorectal atresia/stenosis	1,709	933	568	33.2	20.8
Dandy–Walker malformation or cerebellar hypoplasia (DWM/CH)	392	157	115	29.3	4.2
Choanal atresia	248	106	70	28.2	2.6
Renal agenesis/hypoplasia	357	119	98	27.5	3.6
Omphalocele	687	255	156	22.7	5.7
Hydrocephaly	845	250	186	22.1	6.8
Limb deficiency[b]	1,966	575	411	20.9	15.0
Longitudinal limb deficiency	772	363	271	35.1	9.9
Intercalary limb deficiency	107	40	34	31.8	1.2
Transverse limb deficiency	1,129	194	125	11.1	4.6
Limb deficiency, not specified	44	15	9	20.5	0.3
Holoprosencephaly	285	87	53	18.6	1.9
Diaphragmatic hernia	1,350	328	188	13.9	6.9
Eye defects[c]	1,144	229	158	13.8	5.8
Intestinal atresia/stenosis	1,257	276	148	11.8	5.4
Anotia/Microtia	1,099	336	123	11.2	7.8
Oral clefts	7,138	1,136	647	9.1	23.1
Cleft lip only	1,647	136	69	4.2	2.5
Cleft palate only	2,490	528	290	11.6	10.6
Cleft lip with cleft palate	3,001	472	287	9.6	10.5
Congenital heart defects (CHD)	20,201	3,704	1,724	8.5	63.1

Birth defects	NBDPS-eligible cases	Cases classified as having multiple defects	Cases with at least two NBDPS-eligible defects		
			Number	Percent	Percent of all MCAs[a]
Bladder exstrophy	107	22	9	8.4	0.3
Neural tube defects (NTD)[d]	3,477	469	278	8.0	10.2
Biliary atresia	311	51	22	7.1	0.8
Amniotic band syndrome and limb-body wall complex (ABS-LBWC)	573	76	39	6.8	1.4
Craniosynostosis	2,470	239	125	5.1	4.6
Hypospadias	4,261	396	199	4.7	7.3
Gastroschisis	2,337	203	75	3.2	2.7

[a]Percentage of cases with MCAs with at least two NBDPS-eligible birth defects.

[b]The sub-types of limb deficiencies are not mutually exclusive.

[c]This group includes all eye defects eligible for the NBDPS (ano/microphthalmos, glaucoma and anterior chamber defects, and infantile cataracts).

[d]This group includes all neural tube defects eligible for the NBDPS (spina bifida, encephalocele, anencephaly, and craniorachischisis).

TABLE 3

Multiple congenital anomalies (MCA) patterns with the largest observed-to-expected ratio (top 20%) with at least five affected cases in the National Birth Defects Prevention Study, in descending order of the adjusted observed-to-expected ratio

Adj O:E	# of defects in pattern	Obs	Obs with no other NBDPS defect	Sacral agenesis	Anorectal atresia	Esophageal atresia	Limb deficiency	CHD	Renal agenesis	Eye defects	Choanal atresia	NTD	Oral cleft	DWM/ CH	Anotia/ microtia	Hydrocephaly
106.4	4	6	1	1	1	1	1									
88.6	4	7	3	1	1	1		1								
68.9	3	5	1	1			1		1							
53.0	3	16	5	1	1	1										
49.4	4	5	0	1	1		1	1								
46.5	3	7	2	1	1				1							
41.8	3	21	10	1	1			1								
41.4	3	7	1	1		1	1									
36.5	2	71	20	1	1											
33.2	3	7	6	1	1							1				
33.0	3	10	1	1	1		1									
29.4	4	11	5		1	1	1	1								
29.1	3	11	3		1		1		1							
28.9	3	5	2	1	1										1	
27.6	3	6	4					1		1	1					
22.6	3	7	4					1		1				1		
22.2	3	8	1	1			1	1								
21.1	3	8	0	1		1		1								
20.2	4	6	3				1	1					1		1	
19.7	3	8	2		1	1			1							
18.4	2	9	1	1					1							
17.1	3	44	24		1	1		1								
15.2	3	19	5		1	1	1									
14.9	2	13	3							1	1					
14.2	2	23	5	1			1									
13.6	3	6	5			1		1			1					

Adj O:E	# of defects in pattern	Obs	Obs with no other NBDPS defect	Sacral agenesis	Anorectal atresia	Esophageal atresia	Limb deficiency	CHD	Renal agenesis	Eye defects	Choanal atresia	NTD	Oral cleft	DWM/ CH	Anotia/ microtia	Hydrocephaly
13.6	2	20	9							1				1		
13.5	2	49	25		1				1							
13.5	2	17	4	1								1				
13.0	2	20	1	1		1										
12.9	3	5	0			1							1			1

Abbreviations: Adj O:E, adjusted observed-to-expected ratio; CHD, congenital heart defect; DWM/CH, Dandy–Walker malformation/cerebellar hypoplasia; NTD, neural tube defect; Obs, observed.

TABLE 4

Results from sensitivity analysis after removing syndromic cases

	Results from main analysis *n* = 42			Results from sensitivity analysis *n* = 24		
Combination of MCAs	**Observed**	**Observed with no other NBDPS defect**	**Adjusted O:E ratio**	**Observed**	**Observed withno other NBDPS defect**	**Adjusted O:E ratio**
Anorectal atresia/stenosis + NTD + sacral agenesis	7	6	33.2	3	3	16.4
Anorectal atresia/stenosis + anotia/microtia + sacral agenesis	5	2	28.9	2	2	13.7
CHD + eye defect + choanal atresia	6	4	27.6	3	2	16.2
CHD + eye defect + DWM/cerebellar hypoplasia	7	4	22.6	5	3	17.9
CHD + oral cleft + limb deficiency + anotia/microtia	6	3	20.2	4	3	15.4
CHD + esophageal atresia + choanal atresia	6	5	13.6	3	2	7.5
Oral cleft + esophageal atresia + hydrocephaly	5	0	12.9	4	0	10.8

Abbreviations: CHD, congenital heart defect; DWM, Dandy–Walker malformation; NTD, neural tube defect; O:E, observed-to-expected.

TABLE 5

Clinical characteristics of eligible cases in the National Birth Defects Prevention Study, by presence of MCAs

	NBDPS-eligible cases with isolated defects (*n* = 42,061) *n* (%)	**NBDPS-eligible cases with MCA (*n* = 2,734) *n* (%)**	***p*-value**
Sex			.1035
Male	24,569 (58.4%)	1,544 (56.5%)	
Female	17,212 (40.9%)	1,155 (42.3%)	
Birth outcome			<.0001
Live born	40,378 (96.0%)	2,484 (90.9%)	
Stillborn	715 (1.7%)	111 (4.1%)	
Termination	914 (2.2%)	135 (4.9%)	
Gestational age at delivery[a]			<.0001
<37 weeks	9,396 (23.3%)	1,037 (41.8%)	
≥37 weeks	30,242 (74.9%)	1,420 (57.2%)	
Birth weight[†]			<.0001
<2,500 g	8,431 (20.9%)	1,045 (42.1%)	
≥2,500 g	31,610 (78.3%)	1,434 (57.7%)	
Plurality			<.0001
Single	39,619 (94.2%)	2,513 (91.9%)	
Multiple	2,315 (5.5%)	217 (7.9%)	
Maternal age at delivery			.4741
<20 years	4,992 (11.9%)	325 (11.9%)	
20–34 years	30,926 (73.5%)	2,032 (74.3%)	
35+ years	6,142 (14.6%)	376 (13.8%)	
Maternal race/ethnicity			<.0001
Non-Hispanic White	24,222 (57.6%)	1,355 (49.6%)	
Non-Hispanic Black	4,584 (10.9%)	342 (12.5%)	
Hispanic	10,090 (24.0%)	813 (29.7%)	
Other	2,922 (7.0%)	203 (7.4%)	
Site			<.0001
Arkansas	5,164 (12.3%)	258 (9.4%)	

	NBDPS-eligible cases with isolated defects (*n* = 42,061) *n* (%)	NBDPS-eligible cases with MCA (*n* = 2,734) *n* (%)	*p*-value
California	5,154 (12.3%)	407 (14.9%)	
Iowa	4,277 (10.2%)	256 (9.4%)	
Massachusetts	5,489 (13.1%)	279 (10.2%)	
New Jersey	2,016 (4.8%)	144 (5.3%)	
New York	3,327 (7.9%)	194 (7.1%)	
Texas	4,711 (11.2%)	403 (14.7%)	
Atlanta	4,768 (11.3%)	298 (10.9%)	
North Carolina	2,955 (7.0%)	234 (8.6%)	
Utah	4,200 (10.0%)	261 (9.6%)	
Birth year			<.0001
1997–2001	11,603 (27.6%)	581 (33.7%)	
2002–2006	15,524 (36.9%)	613 (35.6%)	
2007–2011	14,934 (35.5%)	530 (30.7%)	

[a]Gestational age and birth weight distributions include live born children only.