Research Article
Causes of Congenital Malformations

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Background: Many different causes of malformations have been established. The surveillance of a consecutive population of births, including stillbirths and elective terminations of pregnancy because of fetal anomalies, can identify each infant with malformations and determine the frequency of the apparent etiologies. This report is a sequel to the first such analysis in the first 10 years of this Active Malformations Surveillance Program (Nelson and Holmes, 1989). **Methods:** The presence of malformations was determined among 289,365 births over 41 years (1972–2012) at the Brigham and Women's Hospital in Boston. The abnormalities were identified from the review of the examination findings of the pediatricians and consultants and diagnostic testing for the live-born infants and the autopsies of the fetuses in elective terminations and stillbirths. **Results:** A total of 7020 (2.4%) infants and fetuses with one or more malformations were identified with these apparent etiologies in 26.6%: Mendelian disorders, including infants with postaxial polydactyly, type B; chromosome abnormalities; vascular disruption; complications of monozygous twinning; and environmental factors. The malformations of unknown etiology were a much larger group. **Conclusion:** While several causes of malformations have been identified, many remain unexplained. Combining the ascertainment in a future surveillance programs with genome sequencing and chromosome microarray analysis will increase significantly the number of malformations attributed to genetic mechanisms.

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Key words: Mendelian inheritance; vascular disruption; chromosome abnormalities; twinning; environmental factors

Introduction

Population-based malformations surveillance programs have shown that approximately 2% of newborn infants have a structural abnormality or malformation (Correa et al., 2007; Feldkamp et al., 2017). The frequency increases in the first year of life as "silent" abnormalities not detected at birth, such as heart defects, anomalies of the urinary tract, and bowel malrotation, are identified from diagnostic studies prompted by signs and symptoms in the affected infant (Thomas et al., 2018).

The frequency of malformations is much higher in the fetuses in spontaneous abortions and in stillbirths than the 2% in live-born infants. Shepard and his associates (1989) found that 19% of spontaneously aborted fetuses had a localized defect or an identifiable syndrome. In addition, there is a higher frequency of associated chromosome abnormalities in approximately half of spontaneous abortions (Zhang et al., 2009). In an analysis of 789 stillbirths, Pauli and Reiser (1994) found that 34% had single malformations and recognizable malformation syndromes and 25% had chromosome abnormalities.

This report is a follow-up to the compilation of the causes of congenital malformations identified in the surveillance of 69,227 infants, including stillbirths and elective terminations, in the first 10 years of the Active Malformations Surveillance Program, conducted at Brigham and Women's Hospital (BWH) in Boston (Nelson and Holmes, 1989; Holmes, 2012). Several potential causes have been established for malformations during this project extended to a 41-year period (1972–2012): chromosome abnormalities, mutations with autosomal or X-linked inheritance; vascular disruption; multifactorial inheritance/familial; environmental factors, and complications of the twinning process. An additional etiology, vascular disruption, which develops after normal development has occurred, has been delineated as a separate group in the intervening years.

The category multifactorial inheritance was a term used by clinical investigators, such as Fraser (1986) and Carter (1976), for malformations that showed a distinctive pattern of occurrence and recurrence, such as cleft lip and palate (Fraser, 1970) and myelomeningocele (Carter and Evans, 1973). The other common malformations that showed these patterns of inheritance included anencephaly, cleft palate alone, hypospadias, and several common heart defects. Other malformations, such as bilateral renal agenesis (Carter et al., 1979) and esophageal atresia (McMullen et al., 1996), have shown an increased likelihood of occurrence in the siblings of index cases, and have been classified as "familial" in this report.

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Studies establishing the many different causes of a specific malformation highlight the heterogeneity in the etiologies, illustrating the fact that some have a genetic component and others reflect environmental factors. A similar analysis of the causes of malformations was published recently by Feldkamp and her associates (2017). They analyzed 5504 infants with birth defects in a population-based study of 270,878 births in Utah (2005–2009). They assigned a definite cause for 20.2% of the infants with birth defects: 19.1% were chromosomal or genetic conditions and the remainder were due to teratogens, primarily poorly controlled maternal pregestational diabetes.

We present here the apparent etiologies for the 7020 infants with malformations identified by a hospital-based malformations surveillance program among 289,365 births, including live-born and stillborn infants and fetuses from elective terminations because of anomalies.

Materials and Methods

The malformed infants and fetuses were identified 6 days a week and on holidays from reading the findings recorded by the examining pediatricians and the consultants in each newborn infant's medical record. The findings in stillborn infants and affected fetuses in elective terminations of pregnancy were determined from the review of autopsy findings in the Department of Pathology. The final diagnoses were determined from the review of this information by the study clinicians (L.B.H. and A.E.L.). The methodology and the demographic characteristics of the population surveyed by the Active Malformations Surveillance Program at the Boston Lying-In Hospital, later part of the BWH in Boston, have been summarized in another article in this special issue (Holmes et al., 2018).

The reports of the findings in chromosome analysis by the hospital's cytogenetics laboratory were part of the records obtained on each infant tested. Some infants with a diagnosis of a chromosome abnormality from prenatal testing at an outside laboratory had official reports or occasionally only verbal reports.

The common malformations with distinctive patterns of clustering in families, such as cleft palate alone, cleft lip and palate, anencephaly, myelomeningocele, hypospadias, many common heart defects, congenital hip dysplasia, etc., were assigned to the category of multifactorial inheritance. They were combined with other common malformations which have shown an increased rate of occurrence in siblings, such as bilateral renal agenesis, esophageal atresia, and other malformation with an observed increased frequency among the sibs or offspring of affected individuals in comparison to the general population. For this analysis, these malformations have been combined under the heading "multifactorial inheritance/familial."

TABLE 1. *Recognized Causes in Affected Infants*[a]

	1972–74 1979–1985[b] (*n* = 69,277)	1972–74 1979–2012[c] (*n* = 289,365)
Single mutant genes	48 (3.1%)	110 (1.6%)
(Postaxial polydactyly, type B)	[d]	545 (7.8%)
Chromosome abnormalities	157 (10.1%)	825 (11.8%)
Multifactorial inheritance/familial	581 (37.5%)	1461 (20.8%)
Malformation syndromes	N/A	87 (1.2%)
Vascular disruption	39 (2.5%)	105 (1.5%)
Environmental factors	49 (3.2%)	238 (3.4%)
Twinning	6 (0.4%)	36 (0.5%)
Unknown cause	669 (43.2%)	3,613 (51.5%)
Total	1549	7020

[a]Limited to infants born to mothers who had always planned to deliver at BWH.

[b]Published (Nelson and Holmes, 1989).

[c]Findings through 2000, published (Holmes, 2012).

[d]Postaxial polydactyly, type B, was considered "familial" in the report from the first 10 years (Nelson and Holmes, 1989) and has been designated as due to an autosomal dominant gene mutation in the current analysis. Only infants with isolated postaxial polydactyly, type B are listed.

Malformation syndromes, such as VACTERL association and hemifacial microsomia, were compiled as separate from the common isolated malformations.

The designation of vascular disruption has been used for abnormalities that are produced after the initial normal structural development has occurred. In the process of vascular disruption, the hypothesis is that blood flow to a region is decreased, followed by hypoxia, endothelial cell injury, hemorrhage, tissue loss, and repair. This group of affected infants has been presented in detail in a separate article (Holmes et al., 2018).

Many different environmental factors were identified, such as anticonvulsant medication taken to prevent seizures, and maternal conditions, specifically, insulin-dependent diabetes mellitus. However, the ability of a malformations surveillance program to identify these causes of abnormalities in the exposed fetus depends on the accuracy of the information recorded in the medical records of the infants identified as malformed and of their mothers. This is particularly relevant to the identification of an infant affected by exposure to an excessive amount of alcohol or to an intrauterine infection with cytomegalovirus. These infants would be more likely to be identified if the hospital has a screening program with well-designed interviews about alcohol use or routine viral cultures during pregnancy. Because there were no such systematic programs in this setting, the affected infants were much less

likely to be identified at birth. However, several rare teratogenic exposures were identified.

Identical twins have been shown to have a higher rate of malformations than nonidentical twins or singletons (Schinzel et al., 1979). The like-sex twins considered to be identical were based on the findings in the placental membranes, not genetic markers. The abnormalities identified were from pregnancies complicated by twin–twin transfusion and acardia.

Results

The findings in the 41-year sample are very similar to those in the first 10 years (Table 1). The malformations attributed to Mendelian inheritance have been consistent with the findings in Mendelian Inheritance in Man (Amberger et al., 2015). One major difference is that we have tabulated separately postaxial polydactyly, type B, in this analysis and highlight that group of infants, whereas they were included in the "familial" subset in the first 10 years. Because this mild type of polydactyly occurs in 1% of Black infants and only 1 in 3000 White infants, its frequency will reflect the more racially diverse nature of the population surveyed in Boston, in contrast to the analysis by Feldkamp et al. (2017) in the less diverse population in Utah. In the Active Malformations Surveillance Program at BWH, we used a four-level severity score: lethal (anencephaly); severe, handicapping (Down syndrome); moderate, fixable (cleft lip and palate); and mild (postaxial polydactyly, type B). Other malformations surveillance programs may exclude this mild form of polydactyly.

TABLE 2. *Environmental Causes of Malformations*

1. Infants of pregestational diabetic mothers	183[a]
2. Intrauterine infection	
a) Cytomegalovirus	6
b) Toxoplasmosis	2
3. Exposures to teratogens	
a) Fetal alcohol syndrome	12
b) Anticonvulsant drugs[a]	29[b]
c) Misoprostol	2[c]
d) Chorionic villus sampling procedure	1[c]
e) Dilation and curettage procedure	2[c]
f) Anticoagulant warfarin	1[d]
Total	238

[a]Detailed information presented in separate article on the malformations in infants of diabetic mothers in this series of articles: Nasri et al., 2018.

[b]Infants with malformations identified by Surveillance Program; exposures to either monotherapy or polytherapy with valproate, carbamazepine, phenytoin, or phenobarbital.

[c]Infants described in a separate article on vascular disruption in this series of articles: Holmes et al., 2018.

[d]An infant reported in a case series by Hall et al., 1980.

The malformations designated multifactorial inheritance/familial are the largest group (20.8%), but does not represent a single "cause" of malformations. We would expect the abnormalities included in this group to be re-designated in future studies, once new categories have been developed from the on-going research.

The group of malformations designated as due to environmental factors was primarily malformed infants born to mothers with pregestational diabetes mellitus, as was the case for the analysis of the causes of malformations in the population in Utah by Feldkamp et al. (2017) (Table 2). With better prenatal screening and more focused physical examinations, we would have expected to have identified many more malformed infants with the fetal alcohol syndrome. The listing of 29 infants with malformations whose mother had taken one or more anticonvulsant drugs is limited to medications that have been shown to be teratogenic.

Discussion

There were several limitations to this survey of 289,365 births. First, by limiting the information about diagnosis to the first 5 days of life, findings in follow-up studies, such as echocardiogram, a consultation with a urologist, or mutation analysis, were not incorporated. Second, the significant progress made in diagnostic studies between 1972 and 2012, specifically mutation analysis and chromosome microarray, was used in only a few affected infants. More use of these diagnostic methods would have improved the quality and specificity of the diagnostic findings. Whole genome sequencing could have identified even more causative mutations. Third, by the 1980s approximately 20% of the malformed infants were in pregnancies that the parents had chosen to terminate (Peller et al., 2004). These fetuses were likely to have more severe abnormalities (Thomas et al., 2016). The termination procedure makes a diagnostic assessment of that infant's phenotype more limited. Fourth, there were no research study-based examinations of the malformed infants. A study-based exam could have identified important details not identified in routine examinations and led to definitive diagnostic studies. Fifth, it was not possible to preserve DNA obtained from the affected fetuses, stillbirths and liveborn infants.

The evaluations of the many infants with common malformations made it possible to establish the etiologic heterogeneity for several, including myelomeningocele, cleft lip and palate, hypospadias, cleft palate alone, esophageal atresia, and congenital diaphragmatic hernia (CDH) (Holmes, 2012). This experience established the fact that clinicians should expect to identify several different etiologies for common malformations. These diverse causes have practical value. For example, the analysis of limb reduction defects identified a subset which attributed to the process of vascular disruption. This established the baseline prevalence rate which was used to assess whether or not the fetus exposed

to the prenatal diagnosis procedure chorionic villus sampling had a significant increase in the frequency of vascular disruption-type of limb defects (Golden et al., 2003). Another example of the importance of identifying the different causes of a common malformation would be in evaluating infants with neural tube defects. To identify those who could be affected by a preconception supplement with multivitamins and folate, those infants whose abnormality was associated with maternal diabetes mellitus, prenatal exposure to valproate, chromosome abnormalities, and syndromes with Mendelian inheritance should be excluded (Holmes et al., 1976).

Another benefit of an active malformations surveillance program is that the daily searching for each affected infant in both live-born and deceased infants, including stillbirths and elective terminations, can identify uncommon, unplanned, but significant, observations and provide a timely opportunity for documentation. Some examples of these chance observations were: (1) infants in two informative families who died soon after birth and who subsequently had a similarly affected sibling (Holmes et al., 1995, 1997). The collaboration with the pathologists made it possible to determine the phenotypic features and to describe these two "new" hereditary phenotypes; (2) the evaluation of a fetus with severe limb deficiencies made it possible for the pathologist to examine the histologic features of the "nubbins" in a fetus with terminal transverse limb defects with nubbins, a rare opportunity (Drapkin et al., 2003); (3) identifying the rare pregnancy that continued after a dilation and curettage procedure made it possible to evaluate the pattern of abnormalities produced in this seldom-reported clinical setting (Holmes, 1995).

Before the findings reported by Feldkamp and her associates (2017) and this analysis, the frequency of malformations attributed to environmental factors had been estimates by Kalter and Warkany (1983) and Brent (2001). Brent estimated that 10% of malformations observed in the first year of life were due to environmental factors, including maternal conditions, intrauterine infections, and prescription drugs, high dose radiation, and hyperthermia. The frequencies of environmental factors identified by Feldkamp et al. (2017) was 4.1% and in this study, 3.4%. However, it is likely that many newborns with fetal alcohol syndrome were not identified. Some would have had associated malformations and would have been considered to have been caused by environmental factors, that is, prenatal exposure to an excessive amount of alcohol.

Prenatal exposure to anticonvulsant medication is another common exposure that is difficult to prove as having "caused" malformations. Study examinations of infants exposed to valproate, phenytoin, carbamazepine, or phenobarbital could have identified the associated dysmorphic facial features and effects on the digits that would make a causal relationship more likely. Lacking that, the 29 anticonvulsant-exposed infants with serious malformations remain a "likely" causal relationship.

The instructive new findings being developed by chromosome microarray and whole genome or exomic sequencing will add new insights into the recognized causes in infants being born with malformations. Two examples are the progress in evaluating infants with heart defects and infants born with CDHs, abnormalities attributed previously to multifactorial inheritance. In an analysis of 58 infants born with congenital heart defects in 2011 to 2012, 6.7% had abnormalities identified by chromosome analysis. An additional 22.2% had abnormalities identified by chromosome microarray (Bachman et al., 2015). In another study, exomic sequencing of 1213 infants with congenital heart disease (CHD) and their parents identified protein-damaging de novo mutations in 20% of the children with CHD and neurodevelopmental disabilities and in 2% of the children with isolated CHD (Homsy et al., 2015). The second example is from the analysis of DNA from infants with congenital diaphragmatic hernia (Pober et al., 2005) by whole exome sequencing. The recent study identified an excess of damaging de novo gene disrupting and deleterious missense variants in 21% of the infants with multiple malformations including CDH and in 12% of infants with isolated congenital diaphragmatic hernias (Longoni et al., 2017). The de novo variants were more common in genes expressed in the diaphragm and heart of the developing mouse. Similar progress is being made in research studies on a variety of congenital malformations. This progress could revise the classification of the etiologies identified as "causes" of congenital malformations.

CONCLUSIONS

Malformations surveillance programs can delineate the etiologic heterogeneity of the common malformations. This survey of the causes of malformations among 289,365 births over 41 years (1972–2012) identified 7020 (2.4%) infants with one or more malformations. The frequencies of several causes were confirmed: malformations due to mutations with Mendelian inheritance, chromosome abnormalities, complications of identical twinning, environmental factors, and the process of vascular disruption. The new technologies, such as chromosome microarray and genome sequencing, are beginning to identify the genetic abnormalities present in infants with malformations referred to previously as exhibiting multifactorial inheritance.

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