



Deciphering congenital anomalies for the next generation

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Abstract Congenital anomalies are common, with 2%–3% of infants estimated to have at least one major congenital malformation and countless others with minor malformations of lesser cosmetic or medical importance. As congenital malformations are major drivers of morbidity and mortality, representing the leading cause of infant mortality in the United States, there is substantial interest in understanding the underlying etiologies—particularly if modifiable causes may be identified or pre- or postnatal treatments can be offered. Recent research has begun to reveal the spectrum of monogenic disorders that commonly result in birth defects, and newer approaches have revealed non-Mendelian genetic contributions including gene–environment interactions. Our experience suggests that increased efforts to sequence and analyze cases of perinatal death, as well as continued global collaboration, will be essential in understanding the genomic landscape of structural anomalies.

INTRODUCTION

Since the report more than 30 years ago by Nelson and Holmes surveying nearly 70,000 still-born and live-born infants, which established the prevalence of major congenital anomalies in this population at ~2% (Nelson and Holmes 1989), research into the epidemiology and etiology of these anomalies has continued to yield important new insights and reflects the evolution of genetic diagnostic technology. Advances in human genetics and genomics have redefined our understanding of many major structural anomalies occurring in isolation or as part of a syndrome, although the underlying molecular basis of several anomalies—from isolated congenital cardiac defects to commonly seen patterns of malformations such as VACTERL (vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal, and limb abnormalities)—remains poorly understood. Even within a well-defined genetic syndrome, such as Down or Patau syndrome, the variable presentations of structural anomalies remain a mystery (Springett et al. 2015; Stoll et al. 2015).

To further understand these anomalies, collecting detailed and accurate genotype and phenotype data from affected individuals and engaging in open and rich data-sharing initiatives are critically important. Although each major congenital anomaly is individually rare, they are collectively more common and contribute disproportionately to the national and global morbidity and mortality burden (Arth et al. 2017; Boyle et al. 2018; Xu et al. 2020). Current efforts have revealed that many congenital anomalies occur in the context of an identifiable genomic variant, even if many variants remain cryptic to techniques such as chromosomal microarray and exome sequencing (Miller et al. 2010; Lord et al. 2019; Petrovski

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et al. 2019). As genomic technology continues to advance at a tremendous rate, optimizing the impact of new diagnostic techniques will require large-scale collaborations in order to make meaningful discoveries. Although pinpointing the genetic or genomic variants underlying structural anomalies remains challenging, our experience has shown that postmortem sequencing of perinatal deaths is particularly high yield for genetic diagnosis and gene discovery, despite the logistical difficulties. The genomic or molecular “autopsy” will be another key contributor to deciphering congenital anomalies.

SURVEILLANCE FOR CONGENITAL ANOMALIES

Many factors influence the prevalence of congenital anomalies in the general population. The point of ascertainment, as well as the method and even the definition of what constitutes a congenital anomaly, may substantially influence these calculations (Kirby 2017). For example, structural abnormalities are thought to affect 3% of pregnancies (Centers for Disease Control and Prevention 2008), 20% of all pregnancy losses (Heinke et al. 2020), 8.5% of stillbirths (fetal deaths after 20 wk) (Holmes et al. 2018), and 2.4% of live-born infants (Nelson and Holmes 1989; Mai et al. 2019). Furthermore, public health policies may also influence both the prevalence and mortality rates associated with congenital anomalies, as countries that do not allow termination of a pregnancy because of congenital anomalies report higher rates of neonatal mortality caused by these conditions (Boyle et al. 2018).

Congenital anomalies may be detected by physical examination and/or imaging studies. As imaging techniques have improved, “technology-detected malformations” have been identified: asymptomatic structural anomalies that would be detected only by advanced imaging. These anomalies—nonsevere atrial septal defect or ventricular septal defect and patent ductus arteriosus (excluding those found in preterm infants that are physiologically normal)—have increased in prevalence over time compared to anomalies such as tetralogy of Fallot (Straub et al. 2019). As prenatal imaging techniques continue to improve, congenital anomalies may be more frequently identified even earlier in pregnancy. The genetic underpinnings of these more subtle anomalies, compared to severe malformations leading to fetal demise, may differ substantially. Including the entire spectrum in sequencing studies is critical, particularly as defining the full fetal phenotype of genetic syndromes associated with structural malformations is needed to interpret prenatal genetic testing results and for prenatal counseling (Gray et al. 2019).

Identifying cases for prospective studies can be achieved in various ways. Most studies focus on major congenital anomalies, or those of cosmetic or medical importance to the individual (Centers for Disease Control and Prevention 2015), though others may be more inclusive, particularly those using hospital billing or discharge codes, because of poor distinctions between categories of birth defects within the coding system (Holmes and Westgate 2012). In the United States, funding was established in 1996 for the National Birth Defects Prevention Study (NBDPS), which has collected data on nearly 45,000 cases of major congenital anomalies from 10 centers across the nation (Reefhuis et al. 2015). In addition to collecting data from medical records by trained abstractors, which is more accurate than using ICD codes (Holmes and Westgate 2012), the NBDPS interviews eligible families to identify potential contributing factors and has collected buccal swabs for genetic evaluation of the affected individual and parents for more than 20,000 participants (Reefhuis et al. 2015; Jenkins et al. 2019). The rich data produced by this study and other state-led birth defects surveillance programs (Centers for Disease Control and Prevention 2008) continue to inform birth defects research and have led to hundreds of publications in addition to discoveries of modifiable risk factors and public health interventions, such as the use of folic acid to prevent neural tube defects (Lin et al. 2009; Williams et al. 2015). However, these studies may

be biased toward nonlethal phenotypes, which is important in interpreting their results (Heinke et al 2020).

GENETIC CAUSES OF BIRTH DEFECTS

In the landmark report by Nelson and Holmes, 205/1549 (13%) infants with a major malformation had an identifiable genetic cause—either chromosomal (157/1549, 10%) or monogenic (48/1549, 3.1%), although the underlying genes responsible for the monogenic disorders were poorly understood at the time (Nelson and Holmes 1989). In the 30 years since its publication, genes have been identified for all of the suspected monogenic conditions listed in this paper except Moebius syndrome (Nelson and Holmes 1989). The estimate of a monogenic condition underlying 3.1% of birth defects was made with full knowledge that there were likely other genetic causes of birth defects that had not yet been described (Nelson and Holmes 1989).

There are many different ways in which genomic variation can lead to congenital anomalies. Certain anomalies may be caused by Mendelian disorders, in which a disease-causing change at a single locus results in a spectrum of anomalies. Others may be related to genetic factors that are non-Mendelian. These include oligogenic disorders caused by variants in a few genes (Pehlivan et al. 2019); somatic pathogenic variants that occur in a specific tissue and would not be found across all cells of the body (Lim et al. 2015); single-nucleotide polymorphisms (SNPs) increasing the susceptibility to a particular condition; and epigenetic variants affecting the expression of certain genes (Hobbs et al. 2014). Currently, our ability to assess for Mendelian disorders in the clinical setting outweighs the ability to evaluate for non-Mendelian causes of disease, although this is an active area of research (Boycott et al. 2019).

Estimates of the contribution of genetic disorders to structural anomalies must take into account the time point at which these anomalies are ascertained and the genomic technology used. As many structural anomalies lead to pregnancy loss or occur in pregnancies that are not continued (Heinke et al. 2020), the genetic landscape of birth defects in live-born infants may differ from that of infants who do not survive the pregnancy. The population described by Drs. Holmes and Nelson involved live- or stillborn infants and estimated that ~13% of cases with structural anomalies were explainable by a genetic cause (Nelson and Holmes 1989), compared to the 37%–50% described in studies restricted to stillbirths with structural anomalies using exome sequencing (ES) (Vora et al. 2017; Shamseldin et al. 2018; Quinlan-Jones et al. 2019). The methodologies used to evaluate for genetic causes of birth defects must also be considered. Many structural anomalies occur as part of aneuploidy syndromes detectable by karyotype and chromosomal microarray, the latter of which has been shown to have a diagnostic yield of 6% of prenatal cases of structural anomalies (Wapner et al. 2012) and is commonly used clinically for prenatal diagnosis. Massively parallel sequencing technology is often used as the next step if microarray is unrevealing, particularly ES. Recently, two large studies using ES to evaluate pregnancies in which a fetal structural anomaly was detected found a diagnostic yield of ~10%, with the diagnostic yield increasing for fetuses with more than one anomaly (Lord et al. 2019; Petrovski et al. 2019). In the postnatal setting, ES has been used in many large cohorts focusing on a particular defect and identifying a diagnosis in 37.5% cases of craniosynostosis (Miller et al. 2017), 10% for nonsyndromic oral clefts (Basha et al. 2018), and 14% for congenital anomalies of the kidney or urinary tract (van der Ven et al. 2018). Postnatal cohort studies of critically ill infants undergoing ES for genetic diagnosis have found that congenital anomalies associated with genetic syndromes are often diagnosed using this technology (Meng et al. 2017; Gubbels et al. 2019). Genome sequencing, which has the potential to detect variants outside of the protein-coding regions covered by ES in addition to offering improved ability to detect

structural variants and other types of variants undetectable by ES or gene panel testing, has also been used to evaluate for genetic causes of congenital anomalies. Although at this time, the ability to fully analyze and interpret data generated by genome sequencing remains incompletely understood (Lionel et al. 2018; Perenthaler et al. 2019), promising new research into the role of noncoding variants suggests this as an avenue for further research (Richter et al. 2020).

Certain structural malformations may be more indicative of an underlying Mendelian condition than others. Cardiac anomalies such as truncus arteriosus and interrupted aortic arch are known to be associated with 22q11 deletion syndrome, whereas others such as hypoplastic left heart syndrome are less often associated with a genetic syndrome (Li et al. 2017). Open neural tube defects and midline abdominal defects such as gastroschisis observed in isolation have also been low-yield in genomic sequencing studies (Ross et al. 2017; Salinas-Torres et al. 2020). Future research is likely to reveal genomic changes causing such apparently isolated anomalies. For example, one recent, large sequencing study of left-sided cardiac defects identified 27 candidate genes and found their cohort overall to be enriched for de novo loss of function variants (Li et al. 2017). When multiple anomalies occur together, an underlying genetic diagnosis is more likely to be found, as reflected in prior studies using ES for diagnosis (Lord et al. 2019; Petrovski et al. 2019; Gubbels et al. 2020). However, certain combinations seem to be more high-yield than others. The lack of unique facial features (possibly recognizable in the newborn period) or developmental disorders (not recognizable in the newborn period) may suggest that ES is more likely to be non-diagnostic. Although some recurrent constellations of anomalies have been successfully linked to a single gene, such as the spectrum seen in CHARGE syndrome (coloboma, heart defects, atresia choanae, retarded growth and development, genitourinary anomalies, ear anomalies), caused by pathogenic *CHD7* variants, other associations such as VACTERL and OEIS (omphalocele, bladder exstrophy, imperforate anus, spinal defect) complexes are less likely to have a Mendelian disorder identified, particularly if no other syndromic features are present (Meng et al. 2017). Why they continue to occur in combination remains a mystery.

Future studies are likely to further elucidate the complex genetic mechanisms at play in early embryogenesis when these structural anomalies arise, which may defy traditional Mendelian patterns of inheritance. Examples include the digenic inheritance of both a rare and more common variant causing craniosynostosis (Timberlake et al. 2016), somatic variants found to underlie a type of brain malformation that causes intractable epilepsy (Lim et al. 2015), and de novo noncoding variants recently implicated in congenital heart disease (Richter et al. 2020). The formation of consortiums such as the Centers for Mendelian Genomics (Bamshad et al. 2012; Posey et al. 2019), Care4Rare, and FORGE (Sawyer et al. 2016), the Undiagnosed Diseases Network (Ramoni et al. 2017), the Deciphering Developmental Disorders study (DDD Study et al. 2015), and the Gabriella Miller Kids First Pediatric Research Program (<https://commonfund.nih.gov/kidsfirst/overview>) with the resources to sequence thousands of individuals and share data in a collaborative analytic approach has transformed rare disease research and led to the identification of thousands of novel and candidate novel disease genes (Bamshad et al. 2019), many of which involve congenital anomalies.

GENETIC SUSCEPTIBILITY AND ENVIRONMENTAL CAUSES OF BIRTH DEFECTS

In addition to pathogenic genetic variation, many environmental exposures have been linked to major congenital anomalies, such as maternal diabetes (Nasri et al. 2018); maternal medications such as warfarin, phenytoin (Toufaily et al. 2018), and retinoic acid (Lammer

et al. 1985); maternal conditions such as phenylketonuria (Lenke and Levy 1980); and other maternal exposures such as alcohol consumption or infection (Rasmussen et al 2016). Studies such as the NBDPS have attempted to focus on environmental or teratogenic causes of birth defects by excluding cases in which a monogenic or chromosomal disorder is present, as assessed by review of cases by clinical experts (Reefhuis et al. 2015). However, as genetic testing is not pursued for all cases of birth defects, and the most common test performed is chromosomal microarray (which has a relatively low yield, particularly for isolated congenital anomalies), certain cases included in the NBDPS or other surveillance programs may have monogenic causes that would be found by additional sequencing (Toufaily et al. 2018; Jenkins et al. 2019). Even in the absence of a monogenic cause, there is likely a complex interplay between these exposures and genetic risk factors that further studies are likely to address. Furthermore, genomic sequencing has identified critical pathways involved in the development of structural anomalies that may be amenable to environmental influence, such as the detection of pathogenic variants in the NAD synthesis pathway causing VACTERL association in multiple families that may be treatable by niacin supplementation (Shi et al. 2017). This report exemplifies the interaction between genomic sequencing data, functional evaluation with animal models, and correlation to environmental factors that made lead to targeted therapies. As ES has been successfully performed using DNA extracted from buccal swabs provided in the NBDPS (Jenkins et al. 2019), this represents an exciting opportunity to combine an analysis of genetic and environmental factors in a diverse and large data set to further understand the underlying mechanisms of structural anomalies.

EXAMPLES OF MONOGENIC CAUSES OF CONGENITAL ANOMALIES FROM OUR INSTITUTION

The following families were recruited to our Gene Discovery Core protocol within the Manton Center for Orphan Disease Research at Boston Children's Hospital. Informed consent was obtained via our Institutional Review Board-approved protocol. Trio exome sequencing was performed through the Broad Institute Center for Mendelian Genomics using methods as previously described (Wojcik et al. 2019).

1. A 15-mo-old boy was admitted to our institution for surgical management of mitral and tricuspid valve disease. He was born with an atrial septal defect in addition to the valvar abnormalities. The child was noted to have straight eyebrows, a depressed nasal root, micrognathia, and bilateral 3–4 syndactyly on examination by a clinical geneticist. Chromosomal microarray was not diagnostic, and DNA was obtained for ES, which was performed postmortem and revealed a missense variant in *PRKD1* (c.2017G > C, p.Asp673His, ENST0000415220), confirmed to be de novo in the proband and classified as likely pathogenic by American College of Medical Genetics and Genomics (ACMG) criteria (Richards et al. 2015). This gene has been associated with syndromic cardiac disease (Sifrim et al. 2016), although the full phenotypic spectrum remains to be elucidated. Our case helps to define the phenotype of this rare disorder for patients, families, and providers looking for answers; this opportunity would have been lost if postmortem sequencing had not been performed.
2. A 4-mo-old preterm infant was admitted to the neonatal intensive care unit (NICU) at our institution for management of a congenital craniofacial anomaly. At delivery, she was noted to have fusion of her maxilla and mandible. Her jaw was manually separated in the delivery room and she was intubated and she ultimately required tracheostomy placement. Because of progressive narrowing of her oral opening attributed to gingival adhesions, she was transferred to our institution for a craniofacial evaluation and

management of her congenital syngnathism. On examination by the clinical genetics team, she was also noted to have severe micrognathia and “question-mark” shaped ears. She died at 5 mo of age of an unrelated cause, and postmortem trio exome sequencing revealed a de novo missense variant in *PCLB4* (c.1888G > A, p.Asp630Asn, ENST00000378501), classified as likely pathogenic by ACMG criteria, consistent with a diagnosis of auriculocondylar syndrome. This case highlights the challenges faced by children with this condition, as well as allowing counseling for the family on its recurrence risk.

CONCLUSION AND FUTURE DIRECTIONS

Although the last decade has seen impressive advances in our understanding of congenital anomalies and their causes, much remains to be understood. The collection and centralization of high-quality phenotypic data and collaborative sequencing efforts make this an exciting time for research into the causes of congenital anomalies. As genomic sequencing progresses and large data sets are generated for particular birth defects, pooling this data in a publicly available resource for researchers continues to be essential in order to maximize the benefit of these technologies. This coupled with detailed phenotyping and the ability to connect environmental and genetic factors will lead to further breakthroughs. The inclusion of perinatal deaths in these approaches will also be critical to inform our understanding of genomic variants resulting in structural anomalies as the full phenotypic spectrum of many disorders cannot be understood without sequencing these cases.

ADDITIONAL INFORMATION

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Competing Interest Statement

The authors have declared no competing interest.

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