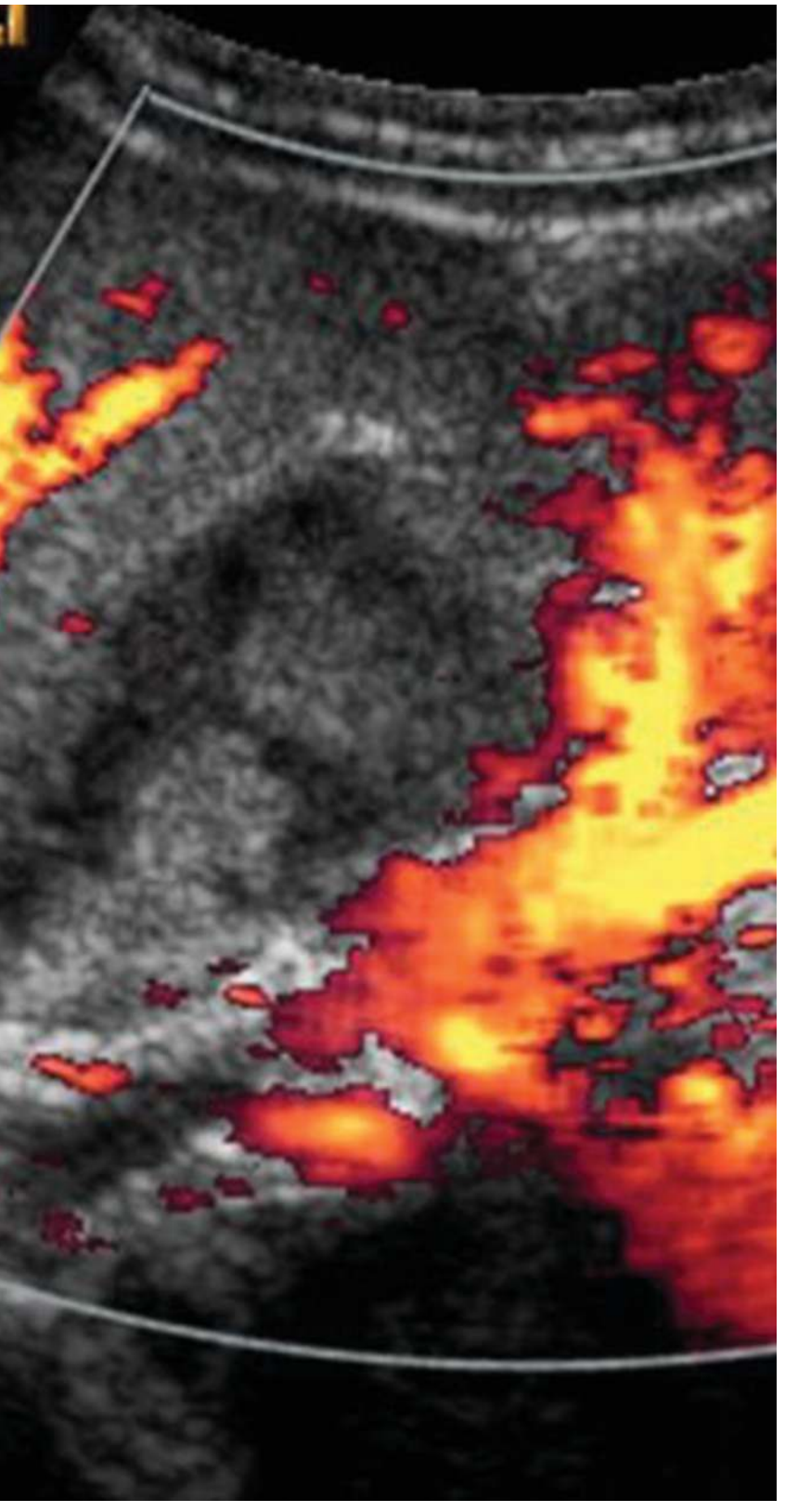
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Decoding Hearts: Genetic Insights and Clinical Strategies in Congenital Heart Disease

Christopher M. Stark, MD, MHS,[1] Brian N. Hughes, DO,[2] John Paul Schacht, DO,[1] Theresa M. Urbina, DO[3]

EDUCATION GAPS

A genetic etiology of structural congenital heart disease (CHD) can be identified in 20% to 30% of cases. However, only half of newborns with CHD are tested for a genetic condition. Clinicians should be aware of prenatal and postnatal diagnostic approaches and recommendations for genetic testing in fetuses/neonates with CHD and how these recommendations vary in cases of isolated and syndromic CHD.

OBJECTIVES *After completing this article, readers should be able to:*

1. Describe the genetic mediators and signal pathways implicated in cardiac embryology.
2. Be able to classify the etiology of a patient's congenital heart disease (CHD) as syndromic or isolated.
3. Identify the implicated genetic mediators of CHD for common syndromes.
4. Recognize the common syndromes associated with structural CHD and their extracardiac manifestations.
5. Explain the role of genetic prenatal and postnatal genetic testing in this population.

ABSTRACT

Structural congenital heart disease (CHD) represents a heterogeneous group of cardiac anomalies of variable embryologic and molecular origins. A basic understanding of the genetics implicated in nonsyndromic (isolated)

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and syndromic structural CHD can better inform management decisions and family counseling. When a fetus or neonate develops CHD as a result of a genetic cause, it can be due to a mutation or a monogenic, oligogenic, or polygenic pathogenic variant. In this review, we summarize basic cardiac embryology in the context of genetic signaling pathways and proteins that are commonly implicated in syndromic and nonsyndromic structural CHD. We also provide an overview of the basic genetic evaluation in infants with common syndromic structural CHD.

INTRODUCTION

Structural congenital heart disease (CHD) affects approximately 1% of all live births and is the most common birth defect in human infants. CHD occurs as a result of disruptions in normal cardiac development causing malformations of the heart, the great arteries, or the pulmonary veins.[1] These malformations can occur in isolation or in combination with other cardiac and noncardiac malformations. Medical teams must tailor their management strategies to the patient's clinical phenotype, taking a distinct approach for fetuses/neonates with extracardiac comorbidities compared with those with isolated CHD.[2]

Nearly 50% to 60% of patients born with structural CHD are diagnosed in utero because of improvements in fetal imaging techniques and more detailed fetal screening of the heart because of the recommendation by The American College of Obstetricians and Gynecologists to include a 4-chamber cardiac view with outflow tracts and a 3-vessel view.[3–5] Rapid advancements and expanded availability of molecular genetic testing over the past decade have increased identification of single and multigenic changes linked to structural heart defects.[6] Epidemiological studies have found that 20% to 30% of patients with CHD have an identifiable genetic cause, with the most common causes being chromosomal anomalies (8%–10%), pathogenic copy number variants (3%–25%), and monogenic pathogenic variants (PVs) (8%).[6]

The overall infant mortality due to CHD has drastically decreased over the past 50 years because of advancements in surgical techniques and approaches.[7,8] As survival improves in this patient population, defining the genetic etiology of a patient's structural CHD is increasingly more relevant and carries clinical implications for the patient, their future siblings, and even their future children. The comorbidities and neurodevelopmental differences associated with CHD will impact these patients as they progress through childhood and onward to adulthood.[9,10]

In this review, we summarize basic cardiac embryology in the context of genetic signaling pathways and proteins that are commonly implicated in syndromic and nonsyndromic structural CHD. We also provide an overview of the basic genetic evaluation in infants with common syndromic structural CHD. The genetics of congenital cardiac conduction anomalies and primary cardiomyopathies are well-described elsewhere and will not be covered in this review.[11–14]

EMBRYOLOGY

The formation of the human heart is a complex embryological process that starts during the third week of gestation with differentiation of the embryonic germ layers (ectoderm, endoderm, and mesoderm) and ends with the folding and division of the primitive heart tube to form the fetal heart. The study of animal embryos has significantly enhanced the understanding of the intricate pathways responsible for cardiac morphogenesis because the genetic pathways involved are largely conserved across species. The interplay between genetic signaling pathways, transcription factors, cell migration and differentiation, programmed cell apoptosis, and fetal blood flow leaves the heart uniquely vulnerable to errors during development. Splanchnic mesoderm cells differentiate into the primitive heart tube, the primary heart field, and an adjacent pool of cardiogenic cells called the secondary heart field.[15] Driven by molecular signaling pathways and transcription factors, the primary and secondary heart fields each contribute cells to the developing heart at discrete time points and at specific locations throughout morphogenesis.[15]

During lateral folding of the embryo, the splanchnic mesoderm of the primary heart field fuses together to form the primary heart tube around day 20 of gestation.[15] Under the control of numerous signaling and transcriptional cascades, the heart tube elongates via the addition of cardiomyocytes from the undifferentiated progenitor cells of the secondary heart field.[15] These cells join the primitive heart tube, leading to the creation of the atria, right ventricle, and outflow tracts. Following elongation, heart tube looping occurs as the primitive ventricle bulges ventrally and moves rightward, whereas the primitive atrium bulges dorsally and to the right and left of the evolving outflow tract (Figure 1).[15] As cardiac looping of the heart tube occurs, many complex events take place to divide the heart tube into 4 chambers with distinct

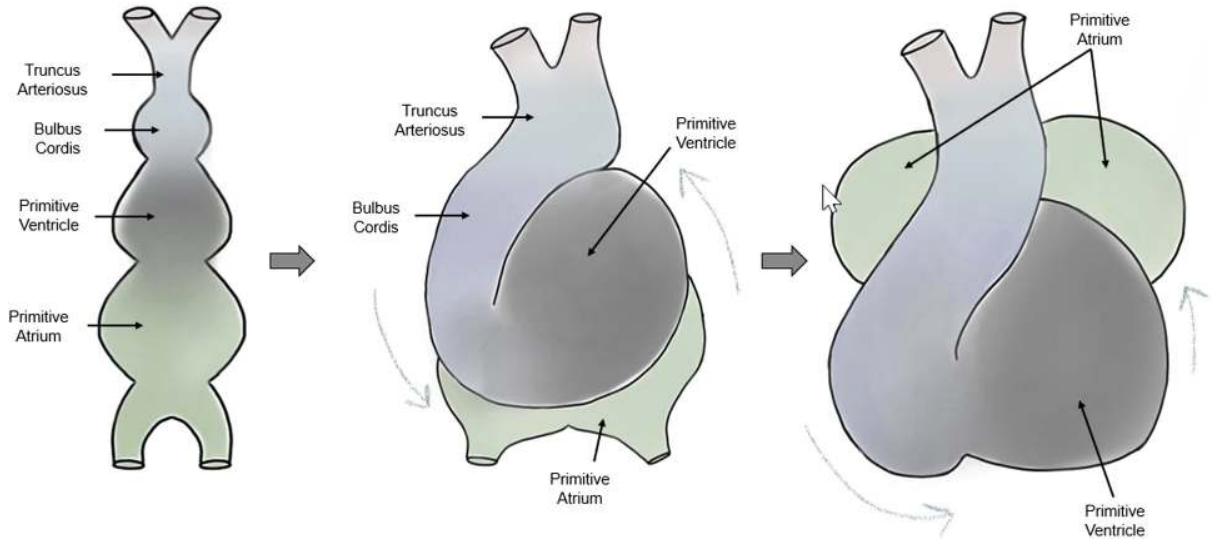


FIGURE 1. View from the ventral side of the embryonic heart during the rotation and folding of the primitive heart tube.

circulations. Table 1 provides a brief summary of select events that occur during normal cardiac development and the involved molecular regulators.

Many of the events detailed in Table 1 occur concurrently over the span of about 5 weeks' gestation as cardiac looping is complete at the end of the eighth week of development as the embryo transitions into a fetus. Developmental diversions during the embryonic period due to errors in signaling pathways, expression or repression of key transcription factors, or cell migration can lead to various cardiac defects. The embryologic origins of structural CHD have been previously described in detail, and a more in-depth review of abnormal cardiac embryology is outside of the scope of this review.[16]

CLINICAL MANIFESTATIONS

Although the majority of CHD cases are sporadic, CHD with an identifiable genetic cause can be broadly categorized as syndromic or nonsyndromic. Genetic changes may be inherited in a Mendelian pattern, which is based on single-gene traits, or a non-Mendelian pattern, which is multifactorial and may involve multiple genes and environmental influences. Patients with syndromic CHD typically have extracardiac manifestations and neurodevelopmental differences (ranging from mild cognitive or processing difficulties to autism spectrum disorder, intellectual disabilities, and global developmental delays). Syndromic CHD is often due to autosomal recessive PVs, de novo monogenic PVs, or chromosomal anomalies.[17–19] Nonsyndromic CHD, on the other hand, is thought to be multifactorial due to the interactions of multiple genes and the environment.[19]

Nonsyndromic CHD

Monogenic PVs often exhibit distinct cardiac phenotypes ranging from septal defects to abnormalities in cardiac chamber development.[20] Monogenic (single-gene), oligogenic (involving 3 or more gene loci), or polygenic (involving many gene loci) PVs have been associated with many CHDs. Although a genetic etiology can be identified in 20% to 30% of patients with CHD, epigenetics and environmental exposures are thought to play a key role as well.[6,15,19,21] Additional risk factors for CHD may include exposures in the pregnant person (eg, valproic acid, retinoic acid), congenital infections (eg, rubella), obesity in the pregnant person, and gestational diabetes (hyperglycemia).[22–24] Importantly, approximately 50% of CHD cases have no identifiable cause.[25]

PVs may disrupt the crucial signaling pathways that govern myocardial patterning, chamber septation, and vascular morphogenesis, which can ultimately lead to disruptions in CHD embryogenesis.[6] Current estimates suggest that there are several hundred single-gene PVs associated with clinically significant structural CHD.[21] Monogenic PVs have been implicated in the disruption of native sarcomeric development, chromatin remodeling, transcription factor regulation, ciliary defects, or other molecular and cellular dysfunction.[26] Although an exhaustive list of single-gene and multigenic PVs is too extensive for this review, Table 2 includes several commonly implicated PVs in isolated CHD. These PVs disrupt cardiac embryologic development, resulting in phenotypic variance depending on penetrance and expressivity. Genetic testing may be completed prenatally or postnatally and consists of multiple modalities, including chromosome analysis, microarray

TABLE 1. Key Signaling Pathways and Transcription Factors in the Embryologic Development of the Heart

	Key Signaling Pathways	Transcription Factor Genes	Cell Origins
Primary heart field	FGF, BMP, TGF-β-Nodal, Wnt, Shh	*TBX5*,[a] *MESP1*, *GATA4*, *NKX2–5*,[a] *MEF2c*, *ISL1*, *HAND1*, *HAND2*	Embryonoic cardiac regions and derived structures
Secondary heart field	FGF, Notch, Retinoic acid,[a] Nodal, Shh	*MEC2c*, *ISL1*, *FOXH1*, *TBX20*, *GATA4*, *NKX2–5*, *TBX1*[a]	
Ventricles[b]			
Right ventricle		*HAND2*, *MEC2c*	Secondary heart field
Left ventricle		*HAND1*,[27] *TBX2*, *TBX5*,[a] *TBX18*	Primary heart field
Interventricular septum		*TBX18*	
Venous pole			
Atria[c]	Wnt	*IRX4*, *GATA6*, *PITX2c*	Primary and secondary (sinus venosus, pulmonary vein, septal and nonseptal myocardium) heart fields
Caval veins			
Pulmonary veins[d]		*NKX2–5*,[a] *ISL1*	
Atrioventricular (endocardial)[e]			
Valves	ErbB (1–4), EGF	*NFATC*	Endocardial cushion
Cushion	BMP2, TGF-β, Notch,[a] FGF, EGF, Wnt, Hedgehog, VEGF	*TBX2*, *TBX3*, *TBX5*, *GATA5*, *NKX2–5*,[a] *MSX2*, *TBX20*, *SOX9*, *ISL1*	Endocardial cells lining the atrioventricular canal
Arterial pole[f]			
Aorta and pulmonary arteries	HAND[28]	*ISL1*, *NKX2–5*, *AP2α*	Neural crest cells, secondary heart field
Semilunar valves[g]	EGF, ErbB1, RAS-Erk,[a] calcineurin, VEGF, Notch		
Right and left ventricular and outlet tracts	Wnt, FGF8/10, BMP, retinoic acid,[a] Shh	*PAX3*, *AP2α*, *HOXa*, *FOXc1*, *FOXc2*, *SOX*, *SLUG*, *TBX1*,[a] *ISL1*	Neural crest cells

Abbreviations: BMP, bone morphogenetic protein; EGF, epidermal growth factor; ErbB, erythroblastosis oncogene B; FGF, fibroblast growth factor; Shh, sonic hedgehog; TGF-β, transforming growth factor β; VEGF, vascular endothelial growth factor; Wnt, wingless-related integration site. This table was adapted from Sizarov et al.

[a]Gene is associated with a known genetic syndrome/defect discussed in the body of this article.

[b]The wall bordering the connection between the trabeculated portions of the primitive right and left ventricles becomes the crest of the ventricular septum, which grows via cell proliferation to divide the inferior portions of the ventricles. The space above the growing muscular septum is remodeled to become the subaortic outlet to the aorta and the right ventricular inlet.

[c]The thin primary atrial septum grows toward the endocardial cushions to divide the common atrium into 2 distinct sides with a patent foramen (or opening) at the superior edge to allow continued connection between the 2 chambers. The larger secondary atrial septum that completes the septum comes from a fold of the right atrial wall rather than a separate and discrete structure.

[d]The wall of the main pulmonary venous stem is absorbed into the roof of the left atrium, leaving each pulmonary vein opening in the 4 corners of the left atrium.

[e]To separate the atria and the ventricles, 2 mesenchymal cushion pairs (1 superior/inferior and 1 lateral) form and enlarge to create the valve primordia. The endocardial cushions eventually fuse with the ridges of the proximal left ventricular outflow tract and right ventricular inlet, becoming the membranous portion of the ventricular septum. The centrally located atrioventricular cushion eventually fuses with the atrial septal mesenchymal cap.

[f]Cardiac neural crest cells migrate into the pharyngeal arches and into the outflow tracts and are responsible for the formation and septation of the great arteries. A pair of cushions are formed at the proximal part of the outflow tract, and cell migration and replication eventually cause these cushions to fuse and separate the 2 spiraling outflow tracts of the aorta and pulmonary artery.

[g]Fetal hemodynamics and blood flow are important for the continued development of the fetal heart.

analysis, single-gene testing, multigene panels, and whole exome or genome sequencing (Table 3). The role of prenatal and postnatal testing will be discussed later in this review.

Syndromic CHD

The noncardiac phenotypic features in an infant with syndromic CHD pose unique diagnostic and management challenges due to the resulting clinical comorbidities. The associated syndromes are characterized by diverse phenotypic expressions necessitating a comprehensive approach to evaluation and clinical care.

Syndromic CHD can be due to chromosomal abnormalities (whole-chromosome aneuploidies and structural abnormalities), copy number variants (duplications or deletions), and monogenic PVs. These genetic changes often arise de novo, although some forms can be inherited. Although a detailed review of every syndrome associated with CHD is beyond the scope of this review, the bedside clinician should have a basic understanding of those commonly seen in practice (Table 4).

Aneuploidies

Down syndrome (ie, trisomy 21) is the leading cause of syndromic CHD. Common cardiac defects seen in Down syndrome include atrial septal defects (ASDs), ventricular septal defects (VSDs), or atrioventricular canal defects.[19]

TABLE 2. Select Monogenic Pathogenic Variants Associated With Cardiovascular Malformations

Gene	Possible Cardiovascular Phenotype(s)	Role(s)
Transcription factors		
GATA4	ASD, VSD, AVSD, PVS, TOF	Regulation of genes involved in embryogenesis and myocardial differentiation[92,93]
NR2F2	AVSD, AS, CoA, VSD, HLHS, TOF	Controls arteriovenous differentiation by regulating cell cycle[94,95]
NKX2–5	ASD, TOF, HLHS, TGA	Regulates heart field development and cardiomyocyte differentiation[96,97]
Cell signaling and adhesion proteins		
ACVR1	AVSD	Endocardial-to-mesenchymal transformation and arterial pole morphogenesis[98]
PDGFRA	TAPVR	Mediates differentiation, migration, and function of mesenchymal cells[99,100]
SMAD6	BAV, CoA, AS	Regulates bone morphogenetic protein and transforming growth factor-β signaling pathways[101,102]
Structural proteins		
DCHS1	MVP	Cadherin acting in the regulation of cell migration involved in valve formation[103]
MYH7	Ebstein anomaly, LVNC	Encodes myosin heavy-chain involved in cardiac muscle development and contractility[104,105]
MYH11	PDA	Encodes myosin heavy-chain involved in cardiac muscle development and contractility[106]
Ciliary components		
NEK2	Heterotaxy	Regulates centrosome separation[107]
EVC2	AVSD	Hedgehog signaling[108]

Abbreviations: AS, aortic valve stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; BAV, bicuspid aortic valve; CoA, coarctation of the aorta; HLHS, hypoplastic left heart syndrome; LVNC, left ventricular noncompaction cardiomyopathy; MVP, mitral valve prolapse; PDA, patent ductus arteriosus; PVS, pulmonary valve stenosis; TAPVR, total anomalous pulmonary vascular return; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

TABLE 3. Overview of Commonly Used Genetic Tests

Test	Primary Uses	Considerations
Chromosomal analysis	Major numerical or structural chromosomal changes	Best test if there is a high suspicion for aneuploidy
Fluorescence in situ hybridization	Aneuploidies or copy number variants	Rapid results Ideal when clinical suspicion for a specific aneuploidy or copy number variant is high, eg: trisomy 13, 22q11.2 deletion syndrome Largely replaced by chromosomal microarray
Chromosomal microarray	Copy number changes (deletions/duplications)	May detect variants of uncertain significance May detect pathogenic variants that are unrelated to the infant's presentation (incidental findings) Cannot detect balanced translocations or inversions (balanced rearrangements)
Targeted gene sequencing	Monogenic variants	Typically used when there is a high suspicion for 1 particular diagnosis May detect variants of uncertain significance
Next generation sequencing Whole exome sequencing Whole genome sequencing	Single nucleotide variants Copy number changes	May detect variants of uncertain significance and incidental findings Costly

Information in this table was adapted from Rosen et al[74] and Gilner et al.[81]

Common extracardiac manifestations include characteristic facial features, poor growth, hypotonia, pulmonary hypertension, and neurodevelopmental differences.[29,30] Most cases of Down syndrome, as well as Edwards syndrome and Patau syndrome, are due to errors in maternal meiosis, with a small subset attributed to balanced Robertsonian translocation. There are many genes involved in cardiac development that are implicated in Down syndrome, including *GATA4*, *CHD7*, and other genes that are involved in the Notch, Wnt, and bone morphogenetic protein (BMP) signaling pathways.[31]

Nearly 45% to 95% of infants born with Edwards syndrome (ie, trisomy 18) will have some form of structural CHD along with typical dysmorphic features.[32] Common structural abnormalities seen in Edwards syndrome include VSD, patent ductus arteriosus (PDA), or ASD. Many genes are implicated in the development of cardiac anomalies in Edwards syndrome including downregulation of some in

TABLE 4. Common Syndromes Associated With Structural Congenital Heart Disease With Their Classic Extracardiac Findings and Common Heart Defects Highlighted

	Classic Extracardiac Features										
Syndrome	**Renal**	**MSK**	**GU**	**CNS**	**FD**	**Eye**	**ENT**	**GI**	**Endo**	**NDD**	**Genetic Testing[a]**
1q21.1[109]				+	+	+				+	CMA
Alagille	+	+		+	+	+		+		+	Targeted, CMA
Cardiofaciocutaneous syndrome[110]		+	+	+	+	+		+		+	CMA
CHARGE syndrome			+	+		+	+		+	+	Targeted, CMA
Char syndrome[111]		+			+						Targeted (*TFAP2*), CMA
Cornelia de Lange[b]		+			+		+			+	Targeted, CMA
Costello syndrome[112,113]		+			+			+		+	Targeted (*HRAS*), CMA
Del1p36[114]				+	+	+	+			+	CMA
Del8p23[c]			+	+	+			+		+	CMA
Del11q24–25 (Jacobsen syndrome)[d]		+					+	+		+	CMA
Del15q11.2[115]				+			+			+	CMA
DiGeorge[e]		+		+	+	+	+		+	+	Targeted, CMA > FISH
Holt-Oram		+									Targeted, CMA
Kabuki syndrome[116]		+			+					+	Targeted (*KMT2D*, *KDM6A*), CMA
Noonan syndrome			+		+		+			+	Targeted, CMA
Rubinstein Taybi[117]		+		+	+	+				+	Targeted (*CREBBP*, *EP300*), CMA
Sotos syndrome[f]	+	+		+	+					+	Targeted (*NSD1*), CMA
Trisomy 9[118]		+	+		+		+			+	Karyotype, CMA
Turner syndrome	+	+	+				+			+	Karyotype, CMA
Patau syndrome		+	+	+	+	+		+		+	Karyotype, CMA
Edwards syndrome		+	+		+					+	Karyotype, CMA
Down syndrome		+			+	+		+	+	+	Karyotype, CMA
Williams syndrome					+				+	+	CMA
Wolf-Hirschhorn syndrome		+	+	+	+		+			+	Karyotype, CMA
Right-sided obstructive lesions	Left-sided obstructive lesions			Conotruncal anomalies			Mixing lesions				Other cardiac defects
ToF - Trisomy 9, Patau, Edwards, DS, Wolf-Hirschhorn, DiGeorge, Del1p36, Del15q11.2, Cornelia de Lange, Alagille, CHARGE Pulmonic atresia Wolf-Hirschhorn, Del15q11.2 Pulmonic stenosis William (branch), Del8p23, Noonan, Alagille, Rubinstein Taybi	Bicuspid aortic valve - Turner, DS HLHS - Turner, Del11q24–25 AS - Williams, Rubinstein Taybi CoA - Turner, DS, Del1p36 IAA - 22q11			DORV - Wolf-Hirschhorn, DiGeorge, 1q21.1 TGA - DiGeorge, 1q21.1 ToF - See right-sided obstructive lesions			AV canal defect - DS, Del3p25.1, Del8p23 ASD or VSD – 1q21.1, CHARGE, Cornelia de Lange, DiGeorge, DS, Del8p23, Del1p36, Del3p25.1, Del15q11.2, Edward, Holt-Oram, Jacobsen, Noonan, Patau, Rubinstein Taybi, Trisomy 9, Turner, Sotos, William, Wolf-Hirschhorn				PDA - Trisomy 9, Patau, Edward, Del1p36, Sotos, Holt-Oram, Cornelia de Lange, Rubinstein-Taybi, Char, CHARGE TAPVR - Del15q11.2 PAPVR – Turner Valvular anomalies - Del1p36, Cornelia de Lange, Cardiofaciocutaneous

Abbreviations: AS, aortic stenosis; ASD, atrial septal defect; AV, atrioventricular; CHARGE, coloboma, heart defects, atresia of the choanae, retarded growth, genital anomalies, ear malformations; CMA, chromosomal microarray; CNS, central nervous system; CoA, coarctation of the aorta; NDD, neurodevelopmental differences; DORV, double-outlet right ventricle; DS, Down syndrome; ENT, ear, nose, and throat; Endo, endocrine; Eye, ophthalmologic anomalies; FD, facial dysmorphisms; FISH, fluorescence in situ hybridization; GI, gastrointestinal; GU, genitourinary; HLHS, hypoplastic left heart syndrome; IAA, interrupted aortic arch; MSK, musculoskeletal; PAPVR, partial anomalous pulmonary venous return; PDA, patent ductus arteriosus; TAPVR, total anomalous pulmonary venous return; TGA, transposition of the great arteries; ToF, tetralogy of Fallot; VSD, ventricular septal defect; WES, whole exome sequencing.

[a]Targeted gene panels available; nonchromosomal syndromes listed can also be diagnosed via next-generation sequencing techniques.

[b]More than 90% of patients have dentition anomalies.

[c]Congenital diaphragmatic hernia.[42]

[d]Including bone marrow failure.[119]

[e]Including immune deficiency.

[f]Classic triad includes characteristic facial features, intellectual disability, and overgrowth.[120]

the T-box family (*TBX1* and *TBX4*) and inhibitors of the BMP signaling pathway (*NOG*).[19]

Patau syndrome (ie, trisomy 13) is associated with major anomalies of multiple organ systems, and most liveborn infants die in the first year after birth.[33] The majority of patients with Patau syndrome are born with a structural CHD, with ASD, PDA, and VSD being the most common. Patients with Patau syndrome have shown gain-of-function PVs in *FOXO1* and other genes that have transcriptional functions in cardiac development.[19]

Turner syndrome (ie, monosomy X) is associated with structural CHD, dysmorphic features, short stature, renal and skeletal anomalies, and neurodevelopmental and behavioral differences. The CHDs in patients with Turner syndrome are typically left-sided obstructive heart lesions such as coarctation of the aorta, bicuspid aortic valve, and others and are associated with haploinsufficiency of the *SHOX* (short-stature homeobox) gene, which is involved in pharyngeal arch development.[34,35] Female patients with a diagnosis of a bicuspid aortic valve or left-sided obstructive lesions should undergo a genetic evaluation for Turner syndrome.[36]

Deletions/Duplications

DiGeorge syndrome, or velocardio-facial syndrome, is due to a loss of DNA in chromosome 22q11, which can affect either *TBX1* or *CRKL*.[37] *TBX1* is expressed in the outflow tracts of the heart and regulates patterning of the inflow-outflow poles because of its role in neural crest cell migration, whereas *CRKL* serves as a modulator of *TBX1*. Mouse models have demonstrated that the balance between *TBX1* function and retinoic acid signaling is vital for normal pharyngeal and conotruncal development.[38] Given that genetic errors in these pathways affect neural crest cell migration and pharyngeal arch development, DiGeorge syndrome is characterized by conotruncal anomalies and extracardiac manifestations related to pharyngeal arch maldevelopment (hypocalcemia, thymic aplasia, and cleft lip or palate).[39]

PVs in another T-box gene, *TBX5*, are implicated in the clinical features of Holt-Oram syndrome characterized by CHD and upper-limb anomalies.[40] *TBX5* plays an important role in atrial septation and endocardial cushion formation (in combination with *GATA4* and *NKX2–5*); thus, PVs have been associated with ASDs and VSDs in this group of patients. Although most PVs in Holt-Oram syndrome arise de novo, they can also be familial with an autosomal dominant inheritance pattern with complete penetrance but variable expressivity.[40,41]

Williams syndrome, which is due to a deletion of the Williams-Beuren critical region on chromosome 7q11.23, is characterized by supravalvular aortic stenosis, neurodevelopmental differences, and connective tissue and endocrine abnormalities. Williams syndrome is most often caused by a de novo deletion but can also be inherited in an autosomal dominant fashion.[42] The supravalvular aortic stenosis found in affected patients is typically progressive and related to an elastin arteriopathy caused by *ELN* deletion.[43]

Deletions in 8p23.1, causing haploinsufficiency in *GATA4* and *SOX7*, are characterized by structural CHD and congenital diaphragmatic hernia.[44] PVs in *GATA4* have been linked to ASDs (most commonly), VSDs, and tetralogy of Fallot, and they are more likely due to the role of *GATA4* in the primary heart field, the early structure that gives rise to much of the heart's left ventricle and atria during embryogenesis.[44] *SOX7* is thought to lie upstream of *GATA4* in the regulatory signaling pathway, as haploinsufficiency of *SOX7* is presumed to result in more severe cardiac phenotypes in patients with *GATA4* deletions (eg, hypoplastic left heart syndrome and pulmonary valve stenosis).[44]

Coloboma, heart defects, atresia of the choanae, retarded growth, genital anomalies, ear malformations (CHARGE) syndrome is an inherited disorder characterized by coloboma, heart defects, atresia of the choanae, retarded growth, genital anomalies, and ear malformations due to PVs in the *CHD7* gene.[45] These PVs frequently arise de novo but can occasionally be inherited in an autosomal dominant manner.[45] The cardiac phenotype in CHARGE syndrome varies widely, with defects ranging from ASDs and PDAs to more severe conotruncal anomalies such as tetralogy of Fallot.[46] *CHD7* plays a critical role in neural crest cell development, contributing to the multiple anomalies observed in CHARGE syndrome, and the location of PVs in different functional domains of *CHD7* leads to distinct cardiac manifestations, emphasizing the genotype-phenotype correlation within this syndrome. Clinical overlap exists between CHARGE, DiGeorge, and Kabuki syndromes due to shared genetic pathways and interactions between their implicated genes. For instance, 2 genes associated with DiGeorge syndrome, *CHD7* and *TBX1*, are mediated through effects on p53. Similarly, Kabuki syndrome results from PVs in genes involved in chromatin remodeling pathways that overlap with those affected by *CHD7*, highlighting why some syndromes share phenotypic similarities.[46]

Single-gene Variants

Cornelia de Lange syndrome is most often due to de novo PVs in 1 of 3 cohesin-associated genes (*NIPBL*, *SMC1L1*, and *SMC3*). It is characterized by distinctive facial features, growth restriction, and hypertrichosis and is often associated with structural CHD.[47] The cardiac anomalies most often

seen in Cornelia de Lange syndrome are pulmonic stenosis, VSD, and ASD and may be due to decreased expression of *NKX2–5* and *MESP1* as a result of PVs in the *NIPBL* gene.[48,49]

Many genes have been implicated in the development of Noonan syndrome, which is characterized by dysmorphic facies, short stature, structural CHD, and neurodevelopmental differences. Pulmonic stenosis (strongly associated with PVs in *PTPN11*) is most common, but patients may also present with tetralogy of Fallot, ASDs, or VSDs. Many genes have been implicated in Noonan syndrome and are inherited in an autosomal dominant fashion, although a minority of patients have de novo PVs.[50] Noonan is one of many RASopathies (including Costello syndrome, cardiofaciocutaneous syndrome, and Noonan syndrome with multiple lentigines) that show shared disruption in the Ras/mitogen-activated protein kinase signal transduction pathway responsible for valvular morphogenesis.[51] In contrast to moderate and severe pulmonic stenosis in Noonan syndrome, milder versions tend to be nonprogressive and often will not require intervention.[52]

Single-gene PVs in *JAG1* and *NOTCH2* are implicated in the CHD seen in Alagille syndrome.[53] Alagille syndrome is characterized by cholestasis due to a paucity of liver bile ducts, butterfly vertebrae, posterior embryotoxon, and structural CHD.[54] Although peripheral pulmonary artery stenosis is the most common CHD described in Alagille syndrome, affecting about two-thirds of patients, other cardiac defects such as tetralogy of Fallot and left-sided obstructive lesions have also been described.[55] *NOTCH2* is involved in cardiomyocyte differentiation into ventricular-like cardiomyocytes and has been implicated in patients with hypoplastic left heart and other outflow tract anomalies.[56] In patients with Alagille syndrome, defects in the Notch signaling pathway lead to disruptions in neural crest cell migration to the sixth aortic arch during embryonic development, which contribute to the pulmonary vasculature.[57] PVs in *JAG1* have also been implicated in families with multiple generations of peripheral pulmonic stenosis in the absence of liver disease.[55] *JAG1* plays a critical role in the Notch signaling pathway and is essential for proper development of the heart's outflow tract and valve formation. Mutations in *JAG1* can lead to a range of CHD phenotypes, including pulmonic stenosis, tetralogy of Fallot, and VSDs.[58]

The syndromes described in this section underscore the connection between genetic aberrations and the resulting disruptions in the molecular regulators of cardiac morphogenesis. Advances in genetic testing have allowed for the identification of specific genetic variations linked to syndromic CHD, providing valuable insights into the underlying pathophysiology.

PRENATAL DIAGNOSIS AND MANAGEMENT

Structural CHD is often first diagnosed at a routine prenatal ultrasonogram anatomy evaluation. When a suspected cardiac anomaly is identified, further imaging with a detailed transabdominal fetal echocardiogram is recommended, optimally between 18 to 22 weeks' gestation.[5] In some cohorts, prenatal echocardiography identified nearly 60% of severe structural CHD, although its sensitivity and specificity vary depending on the gestational age of testing.[59] If CHD is suspected, a detailed anatomical survey must be completed to identify the presence of extracardiac congenital anomalies. Results from a complete fetal ultrasonogram and detailed family history identifying first- or second-degree relatives with a history of CHD can help genetic specialists target recommendations for genetic testing.

Unfortunately, not all patients with structural CHD will be identified in the prenatal period because of multiple factors. Hemodynamics and blood flow play an important role in heart development, and a routine prenatal anatomy evaluation may miss cardiac defects that worsen as the fetus grows (ie, severe valvular stenosis). In addition, pulmonary vascular abnormalities are often not identified in the prenatal period because of limited pulmonary blood flow in utero. Advances in imaging technology, including the use of fetal cardiac magnetic resonance imaging, may soon be more widely adopted to augment fetal echocardiogram and increase rates of prenatal detection of CHD.[60]

Although an anatomy scan and fetal echocardiography are the best available tests for prenatal CHD screening, they cannot definitively differentiate between nonsyndromic and syndromic diagnoses. Prenatal genetic testing, fetal ultrasonogram findings, and serum biomarker screening in the pregnant person are standard components of prenatal obstetric care. Like prenatal echocardiography, prenatal genetic testing has limitations based on the type of test completed.

Cell-free fetal DNA (cffDNA) testing detects the presence of fetal DNA shed by placental trophoblasts into the blood circulation of the pregnant person. This test is performed on the pregnant patient's blood sample obtained at 9 to 10 weeks of gestational age to ensure detection of fetal cells in the circulation of the pregnant person. cffDNA testing is limited in scope because of the relative instability of fetal DNA.[61,62] Aneuploidy (eg, trisomies 13, 18, 21) is the earliest identifiable genetic cause of CHD detected by cffDNA and has a greater than 99% negative predictive value depending on risk stratification in the pregnant person.[63]

If high suspicion exists for syndromic CHD, then more extensive genetic testing should be offered to the family. Amniocentesis allows for chromosomal analysis, microarray evaluation, and monogenic testing (including multigene

panels and exome sequencing) and is preferably done between 15 to 20 weeks' gestation, although it can also be performed later for families desiring a prenatal diagnosis.[64] Chorionic villus sampling (CVS) allows for chromosomal analysis and microarray evaluation of fetal cells. CVS may also be used to evaluate for monogenic conditions via gene panels and exome analysis. CVS is preferably completed before 13 weeks of gestation and may not be feasible if the diagnosis occurs during the standard screening ultrasonogram.[65]

Family Counseling

If CHD is suspected or identified based on prenatal imaging, counseling becomes a crucial component to guiding families through the complex perinatal period and decision-making process. In cases of an isolated and uncomplicated cardiac lesion with no known or suspected family history of CHD, genetic testing can be deferred and reconsidered after birth if new concerns arise in the postnatal period. In the setting of a suspected syndrome, prenatal genetic testing may confirm a suspected diagnosis and facilitate risk stratification, which allows clinicians to provide more informed counseling to aid in a family's decision-making regarding their pregnancy.

A prenatal diagnosis of CHD has a significant impact on a family and the pregnancy. A prenatal diagnosis of CHD is associated with significant familial psychological stress.[66] Pregnancies affected by fetal CHD have a higher incidence of preterm birth compared with pregnancies unaffected by CHD (23% vs 10%, respectively).[67,68] Pregnancies affected by fetal CHD have a higher risk of pregnancy loss (5.3%; 95% CI, 3.7–7.6) compared with the general population (0.6%; adjusted relative risk [aRR], 9.0; 95% CI, 6.0–13.0). Furthermore, 1.4% of pregnancies with isolated (ie, nonsyndromic) fetal CHD resulted in pregnancy loss, with an aRR of 2.0 (95% CI, 1.0–6.0) compared with the general population.[69] Extracardiac anomalies, genetic diagnoses, or valve regurgitations identified prenatally further exacerbate this risk.[70] In addition, in cases of syndromic or inoperable fetal CHD (eg, hypoplastic left heart syndrome with nutmeg lung), some families may elect to pursue pregnancy termination or comfort care following delivery.[71]

Because of the potentially complex decisions affected families may face, a multidisciplinary approach is crucial. Maternal-fetal medicine specialists, genetic counselors, pediatric cardiologists, and neonatologists should collaborate to provide comprehensive information about the nature of the suspected condition, potential outcomes, and treatment options. Emotional support should be offered to allow families time and space to grieve the loss of previous expectations for their pregnancy. Decisions regarding further testing should be discussed on a timeline that considers both the family's emotional readiness, the constraints of diagnostic tests, and the availability or restrictions of pregnancy termination, if desired. The presence of genetic and extracardiac anomalies are associated with a greater likelihood to pursue pregnancy termination.[72,73] Families should also be informed of the indications, risks, and benefits of additional testing. cffDNA testing poses no risk to the developing fetus but is limited in its diagnostic capabilities, whereas amniocentesis and CVS have greater diagnostic capabilities but present a small risk of fetal injury, miscarriage, or preterm birth.

Effective risk counseling is a crucial aspect of prenatal care, ensuring families are well-informed about potential complications and outcomes related to their pregnancy. All families should receive counseling and support regarding the risks associated with their pregnancy, including premature delivery, prolonged infant hospitalizations, and any anticipated long-term outcomes.[74] An accurate, early diagnosis with evidence-based information regarding the prognosis and the expected disease course of a fetus with syndromic and nonsyndromic structural CHD is important, and clear documentation is paramount.

POSTNATAL DIAGNOSIS AND MANAGEMENT

Postnatal transthoracic echocardiography is noninvasive and offers real-time assessment of cardiac anatomy and function, with a high sensitivity and specificity for diagnosis of structural CHD.[75] Prenatally diagnosed cardiac lesions should be confirmed postnatally on a timeline commensurate with the clinical significance of the lesion to allow for a more complete cardiac evaluation. If prenatally identified cardiac lesions are concerning for ductal dependency or physical examination findings are concerning for a genetic syndrome, echocardiography should be completed shortly after birth and a genetic evaluation, if clinically indicated, should be coordinated before hospital discharge.

Understanding differences in nonsyndromic and syndromic structural CHD management is crucial to optimizing outcomes. It is appropriate to perform a screening echocardiogram for patients with dysmorphic features or multiple extracardiac anomalies suggestive of an underlying genetic syndrome. Echocardiography should be performed, even if the prenatal echocardiogram was unremarkable, as not all CHDs can be detected prenatally. In an infant with prenatally diagnosed CHD and multiple anomalies present at birth, an anatomic survey may be considered. Diagnostic studies to consider, depending on the suspected phenotype, include a postnatal echocardiogram, ophthalmologic examination, skeletal survey, and ultrasonographic evaluation of the abdomen, kidneys, brain, and spine.[76] A clinical

geneticist or genetic counselor can further guide clinical evaluation depending on the dysmorphisms on examination and imaging. Results of anatomic survey and subsequent laboratory evaluations assist the selection of high-yield genetic tests to improve overall diagnostics and prognosis.

Postnatal Genetic Testing

Recommendations for genetic testing for isolated structural CHD vary based on the lesion, clinical course, and family history. Routine genetic testing is generally not required for isolated lesions without a family history suggestive of variability. Some cardiac lesions, such as hypoplastic left heart syndrome, are more often associated with a familial history of CHD, increasing the pretest probability of an identifiable genetic diagnosis.[77] In cases of nonsyndromic CHD with a concerning or unknown family history, testing for genetic etiologies via a multigene panel or chromosomal microarray is indicated.[78] The American College of Medical Genetics maintains broad recommendations for genetic testing, including whole exome or whole genome sequencing, in pediatric patients with complex CHD or other systemic developmental abnormalities.[79] A chromosomal microarray is recommended in any infant with 2 or more major anomalies (eg, structural CHD, neural tube defects), multiple minor anomalies, or growth restriction with congenital anomalies.[76,80]

Care must be taken to select appropriate patients for testing to ensure a cost-effective approach that minimizes the risk of false-positive results that may increase stress for the family.[81,82] Whole exome sequencing has emerged as an important follow-up test in scenarios where multigene panels are negative despite family history or physical examination features suggesting a genetic etiology. Trio testing, in which the child and both biological parents are tested, may assist with the interpretation of whole exome or whole genome results. The identification of single-gene PVs may facilitate characterization of additional phenotypes associated with specific genetic findings, with implications on future disease screenings and developmental support. These results may also help inform families regarding future pregnancies and family planning.

When an infant is suspected of having syndromic CHD, cytogenetic testing (eg, microarrays), exome sequencing, or a multigene panel with an optional follow-up with whole exome sequencing should be offered to the family.[79] If a chromosomal microarray is indicated, the families must receive appropriate pretest and posttest counseling by a trained geneticist, a genetic counselor, or another provider with expertise in interpreting genetic tests.[83] Negative genetic testing results do not necessarily preclude an underlying genetic cause such as intronic or other variants.

If a genetic etiology is suspected and confirmatory postnatal genetic testing is desired, umbilical cord or neonatal blood samples should be collected.[84] Although laboratory requirements may vary, genetic testing often requires large quantities of blood, ranging from 1 to 2 mL for each laboratory test. Placental blood samples, which contain fetal blood, should be collected whenever possible: 2 lithium heparin (typically green caps) tubes for chromosome analysis and microarray and 1 EDTA (typically purple caps, but there is more variation and can be test/laboratory-specific) tube for a multigene panel or microarray.

The increased use of exome or genome sequencing as a tool for identifying causal PVs has led to an increase in the identification of secondary or incidental findings for phenotypes or diseases beyond the original intention of the test.[85] The American College of Medical Genetics regularly releases an updated gene list of "medically actionable secondary findings" and recommends the possibility of sharing this information with the geneticist for subsequent discussion with the family at the time of consent to ensure an informed decision is made regarding their disclosure.[86]

Prognosis

Syndromic and nonsyndromic structural CHD outcomes can vary significantly, reflecting the diverse underlying genetic and clinical factors associated with each category. Individuals with syndromic CHD, including those with Down syndrome or DiGeorge syndrome, face additional health challenges beyond their cardiac anomalies. The presence of multiple congenital anomalies contributes to a more complex clinical course, which is associated with prolonged hospitalizations and variable long-term outcomes. For example, in a Korean retrospective cohort study comparing patients with and without Down syndrome who underwent total correction for CHD, hospital length of stay was significantly longer for the Down syndrome cohort (14 days vs 11 days; $P < .0001$).[87] Individuals with nonsyndromic CHD may experience more favorable outcomes in terms of overall health and neurodevelopment.[10]

The Pediatric Cardiac Genomics Consortium, formed in 2009 by the National Heart, Lung, and Blood Institute, is a research network focused on congenital or acquired heart disease in children to accelerate clinical translation of CHD-related investigations.[88] Such research networks should expand to create open lines of communication to allow rapid dissemination of rigorously tested diagnostic tools and new information. Additionally, ongoing research in medical and surgical management in pediatric CHD patients has contributed to improved outcomes in both populations. Recent advances in survival have highlighted

a knowledge gap in understanding long-term outcomes in these populations and focus areas of ongoing research efforts to ultimately improve quality of life and minimize comorbidities.[89] Research shows that physician and family priorities for complex patients can be incongruent.[90] Researchers must include family members and older children with syndromic CHD in research discussions to create relevant methods and evaluations for primary stakeholders.

Individuals with structural CHD may face challenges related to cardiac function, exercise tolerance, and potential late-onset systemic complications, which are poorly understood.[91–93] Comprehensive multidisciplinary follow-up care, including pediatric cardiologists and developmental pediatricians, is crucial to addressing evolving health care needs of these patients. Longitudinal monitoring, risk stratification, and patient education may enhance the quality of life for individuals with CHD as they grow into adulthood.[10]

CONCLUSION

Newborn hospitalists, neonatologists, and pediatric cardiologists are responsible for the initial diagnosis and management of infants with structural CHD after delivery. Syndromic and nonsyndromic structural CHD may result from monogenic, oligogenic, or polygenic PVs that disrupt normal embryogenesis. Structural CHD is increasingly identified prenatally with fetal echocardiography, which has led to an increasing role for prenatal and postnatal genetic testing. In appropriate clinical scenarios, genetic testing can assist with acute or long-term management, prognostic understanding, and future family planning. A basic understanding of the genetic basis of these complex diagnoses will assist with appropriate management and disposition and improve the understanding of long-term prognosis.

American Board of Pediatrics Neonatal-Perinatal Content Specification

- Know the pathophysiology, including genetics, of a cyanotic neonate.
- Know the anatomy and pathophysiology, including genetics, of a neonate with a left-sided cardiac obstructive lesion
- Know the anatomy and pathophysiology, including genetics, of a neonate with a right-sided cardiac lesion

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NEO QUIZ

1. Rapid advancement and expanded availability of genetic testing has increased the identification of genetic changes linked to structural congenital heart disease (CHD). About 20%-30% of infants with CHD have an associated genetic cause, with chromosomal abnormalities being the most commonly identified genetic factor. Up to what percent of these patients have a chromosomal abnormality?
   A. <1%
   B. 5%
   C. 10%
   D. 15%
   E. 20%

2. The human heart is the first functional organ in utero and its formation follows a complex multistage development process that occurs early in gestation. Cardiogenesis starts with the primary heart tube being formed around day 20 after fertilization and ends with cardiac looping which divides the heart tube into 4 chambers with distinct circulations. At which week of gestation is the process of cardiac formation completed?
   A. 3rd week
   B. 5th week
   C. 8th week
   D. 11th week
   E. 15th week

3. A term infant is admitted to the neonatal intensive care unit (NICU) for further management of cleft lip and palate. Further evaluation demonstrates hypocalcemia, thymic aplasia, and a structural heart defect. Given this constellation of findings, which of the following cardiac defects would be expected?
   A. Atrioventricular canal defect
   B. Bicuspid aortic valve
   C. Coarctation of the aorta
   D. Total anomalous pulmonary venous return
   E. Transposition of the great arteries

4. It is known that select monogenic pathogenic variants are associated with cardiovascular malformation. In particular, GATA4 is a transcription factor known to regulate genes involved in embryogenesis and myocardial differentiation. Of the following possible phenotypes, which is NOT regulated by GATA4?
   A. Atrial septal defect
   B. Hypoplastic left heart syndrome
   C. Pulmonary valve stenosis
   D. Tetralogy of Fallot
   E. Ventricular septal defect

5. An infant born at 38 weeks' gestation is admitted to the NICU for evaluation of structural heart disease. In consultation with genetics, it is determined that a test looking to detect a single nucleotide variant would be best. Which of the following studies is best to detect this type of underlying genetic abnormality?
   A. Chromosomal analysis
   B. Chromosomal microarray
   C. Fluorescence in situ hybridization
   D. Targeted gene sequencing
   E. Whole exome sequencing

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Effects of Assisted Reproductive Technology on Genetics, Obstetrics, and Neonatal Outcomes

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EDUCATIONAL GAPS

This literature review aims to evaluate adverse outcomes related to the use of assisted reproductive technology, particularly neonatal genetics, imprinting disorders, abnormal placentation, and birth defects. Ethical considerations, neonatal intensive care unit admission rates, and peripartum morbidity are also addressed. Our goal is to provide a current perspective on limitations and improvements with assisted reproductive technology and their associated pregnancies.

OBJECTIVES *After completing this article, readers should be able to:*

1. Describe the perinatal and obstetric outcomes related to the use of assisted reproductive technology.
2. Explain the neonatal genetic conditions associated with assisted reproductive technology.
3. Identify the ethical considerations and long-term outcomes related to assisted reproductive technology.

ABSTRACT

The prevalence of infertility has increased in the United States over the past decade with 1 in 5 reproductive-aged women suffering from this diagnosis. The use of assisted reproductive technology (ART) to achieve pregnancy has correspondingly steadily increased. After examining the outcomes of ART births, clear trends of increased preterm birth rate, higher-order multiples, and imprinting disorders have been established among ART-related outcomes. However, the relationship between ART and birth defects, abnormal

ABBREVIATIONS

aHR	adjusted hazard ratio
aOR	adjusted odds ratio
ART	assisted reproductive technology
AS	Angelman syndrome
ASRM	American Society for Reproductive Medicine
BWS	Beckwith-Wiedemann syndrome
CDC	Centers for Disease Control and Prevention
ICSI	intracytoplasmic sperm injection
IVF	in vitro fertilization
MOSART	Massachusetts Outcomes Study of Assisted Reproductive Technology
NICU	neonatal intensive care unit
OR	odds ratio
PGT	preimplantation genetic testing
PGT-A	preimplantation genetic testing for aneuploidy
PGT-M	preimplantation genetic testing for monogenic disorders
PGT-SR	preimplantation genetic testing for structural rearrangement
PR	prevalence ratio
PWS	Prader-Willi syndrome
RSS	Russell-Silver syndrome
SART	Society for Assisted Reproductive Technology

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placentation, and stillbirth require further investigation. This review aims to highlight current literature surrounding ART and its relationship with key obstetrical outcomes, neonatal outcomes, and medical genetics.

INTRODUCTION

Over the past decade, infertility rates have risen among reproductive-aged adults. The World Health Organization states that 1 in 6 adults experience infertility worldwide.[1] In the United States, 1 in 5 reproductive-aged women are unable to achieve a pregnancy within 12 months of trying to conceive.[2] In addition, 1 in 4 women of reproductive age have difficulty getting pregnant or carrying a pregnancy to term.[2] Correlating with rising infertility rates among US women is an equivalent increase in the frequency of infants born as a result of assisted reproductive technology (ART). The Centers for Disease Control and Prevention (CDC) reported 97 128 births from ART in 2021, which is approximately 2% of all infants born in the United States.[3]

Unfortunately, adverse outcomes have been reported for infants conceived by ART. Studies examining singleton pregnancies as a result of ART cycles demonstrated an increased incidence of preterm birth (10.9% [95% CI, 10.0–11.8]), low birth weight (8.7% [95% CI, 7.4–10.2]), small-for-gestational-age status (1.1% [95% CI, 0.9–1.3]), and congenital malformations (5.7% [95% CI, 4.7–6.9]).[4] In 2021, the CDC reported that of the singleton infants born from single-gestation pregnancies, 17.8% were born preterm and 7.9% had low birth weight.[3] However, most studies examining adverse outcomes compared the subfertile population with non-subfertile populations, which is an inappropriate comparison group given underlying health conditions associated with many women with subfertility (eg, polycystic ovarian syndrome, premature ovarian insufficiency, genetic disorders) are confounders and not always addressed in studies, which may lead to selection bias or recruitment bias. Therefore, it is difficult to interpret the true associations between ART and neonatal outcomes.[3] The purpose of this review is to provide a narrative summary about the association between ART, medical genetics, and obstetrical and neonatal outcomes (Table 1).

FEDERAL REPORTING AUTHORITY AND STATE REGULATIONS

The Fertility Clinic Success Rate and Certification Act was passed by Congress in 1992 requiring that all ART cycles performed in the United States be reported to the CDC via the National ART Surveillance System. The establishment of this system has allowed for large-scale regulation and analysis of success rates as well as study of infant outcomes from ART cycles. Reporting requirements for ART clinics are updated regularly via Federal Register Notices, ensuring ongoing regulation of ART practices, consistent data collection, and

TABLE 1. Summary of Key Findings

Topic	Summary Statement
Multiple gestations	IVF pregnancies with higher gestations are associated with a higher risk of preterm birth and low birth weight when compared with IVF singletons (aOR, 11.84 [95% CI, 10.56–13.27]; and aOR, 10.68 [95% CI, 9.45–12.08], respectively).
Prematurity	There has been an increased risk of prematurity (aOR, 1.43 [95% CI, 1.11–1.83]) with use of donor oocytes.
Genetics	The use of ART has been associated with increased incidence of sex chromosome aneuploidies and imprinting disorders. However, there are mixed reports on the effects of ART on de novo mutations, which require further evaluation.
Imprinting disorders	Children conceived via IVF, or IVF/ICSI, appear to be at a higher risk of any imprinting disorder than children conceived naturally (OR 3.67 [95% CI, 1.39–9.74]). Beckwith-Wiedemann syndrome is the most common imprinting disorder associated with ART (relative risk, 5.2 [95% CI, 1.6–7.4]).
Birth defects	Most studies indicate an increased risk of birth defects with IVF/ICSI, although the overall risk remains low (approximately 3.6% vs 3% in the general population).
Stillbirth	Most studies have shown that there is an increased risk of stillbirth (defined as intrauterine death occurring at or above 20 weeks' gestation) with ART pregnancies compared with natural conception (OR, 1.82 [95% CI, 1.37–2.42]).
Peripartum morbidity	Studies have shown that ART pregnancies are associated with abnormal placentation, need for cesarean section (elective and emergent), and postpartum hemorrhage.
NICU admission	There are mixed reports on the effects of ART on NICU admissions, which require further studies to determine any associations.
Long-term outcomes	There is an increased risk of childhood obesity associated with ART conception (aHR, 1.14 [95% CI, 1.06–1.23]). There is also an increased risk of type 1 diabetes in ART-conceived pregnancies from frozen-embryo transfers compared with natural conception (aHR, 1.41 [95% CI, 1.05–1.89]). However, several systematic reviews have found no differences on language development, behavior, social functioning, educational outcomes, or psychological disturbances for ART-conceived pregnancies.

Abbreviations: aHR, adjusted hazard ratio; aOR, adjusted odds ratio; ART, assisted reproductive technology; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; NICU, neonatal intensive care unit; OR, odds ratio.

assurance of the continued integrity of ART. While a small number of clinics do not report to the CDC, 453 clinics currently report data to the National ART Surveillance System, capturing more than 95% of all ART cycles in the United States.[3,5] The Fertility Clinic Success Rate and Certification Act has also mandated a regulatory model for embryology laboratory certification at the state level.

In conjunction with CDC efforts, the Society for Assisted Reproductive Technology (SART) organization further upholds standards of ART. More than 95% of ART cycles in 2021 were completed at SART-affiliated in vitro fertilization (IVF) clinics. The primary objectives of SART include reporting IVF outcomes, setting guidelines for best practices and quality assurances in the field of ART, and advocating for patients. SART is the only medical organization to implement advertising guidelines to regulate accurate clinic self-reporting of successful IVF outcomes, allowing for patients to make informed choices.

ART AND MULTIPLE GESTATIONS/PREMATURITY

Historically, the use of ART adversely impacted perinatal outcomes due to an increase in multiple gestations. In 2021, the American Society for Reproductive Medicine (ASRM) issued guidelines to promote singleton gestations and reduce the likelihood of twin gestation and higher-order gestations (>3 or more fetuses in 1 pregnancy) (Table 2). Multiple gestations have increased risks for complications such as stillbirth, preterm birth, and low-birth-weight infants.[6] Although the incidence of higher-order gestations has significantly declined, the incidence of twin gestations remains relatively higher with ART.[6] Luke et al assessed that 21% of twins and 52% of higher-order multiples are due to non-IVF ART (eg, ovulation induction, artificial insemination, and intrauterine insemination).[7] The decline in higher-order gestations via IVF is attributed to changes in SART guidelines preferencing single-embryo transfers whenever possible. Improved methodologies in IVF, such as use of preimplantation genetic testing (PGT), improved vitrification techniques, and development of culture media allowing for blastocyst transfers, has yielded improving rates of singleton gestations.[7] In an ASRM Committee Opinion issued in 2021, IVF programs are encouraged to track prognostic factors, such as the patient's age, prognosis, and age of the donor oocyte.[6] This aids to guide clinicians on the number of embryos to be transferred to achieve a successful pregnancy, with the goal of mitigating associated adverse outcomes with multiple gestations.[6]

Twins and higher-order gestations conceived via IVF are at a higher risk of adverse perinatal outcomes compared with

TABLE 2. Recommendations for the Limit to the Number of Embryos to Transfer

	Age			
Prognosis	**<35 y**	**35–37 y**	**38–40 y**	**41–42 y**
Cleavage-stage embryos				
Euploid[a]	1	1	1	1
Other favorable[b]	1	1	≤3	≤4
Embryos not euploid[a] or favorable[b]	≤2	≤3	≤4	≤5
Blastocysts				
Euploid[a]	1	1	1	1
Other favorable[b]	1	1	≤2	≤3
Embryos not euploid[a] or favorable[b]	≤2	≤2	≤3	≤3

Justification for transferring additional embryos beyond recommended limits should be clearly documented in the patient's medical record.
[a]Demonstrated euploid embryos, best prognosis.
[b]Other favorable = any 1 of these criteria: fresh cycle (expectation of 1 or more high-quality embryos available for cryopreservation or previous live birth after a prior transfer with sibling embryo[s]); frozen embryo transfer cycle (availability of vitrified day 5 or day 6 blastocysts, euploid embryos, first FET cycle, or previous live birth after an in vitro fertilization cycle).
Adapted with permission from the American Society for Reproductive Medicine.[6]

IVF singletons. The Massachusetts Outcomes Study of Assisted Reproductive Technology (MOSART) conducted an analysis on maternal-child health using clinical IVF data between 2004 and 2008 from the SART Clinical Online Reporting System longitudinally linked to Massachusetts vital records and administrative data.[7] IVF pregnancies with higher-order gestations were associated with a higher risk of preterm birth and low birth weight when compared with IVF singletons (adjusted odds ratio [aOR], 11.84 [95% CI, 10.56–13.27]; and aOR, 10.68 [95% CI, 9.45–12.08], respectively).[7] In addition to preterm birth and low birth weight, there are increased risks for pregnancy-induced hypertension (aOR, 1.43 [95% CI, 1.14–1.78]) and prematurity (aOR, 1.43 [95% CI, 1.11–1.83]) with use of donor oocytes.[7] While the factors underlying these associations are not known, practitioners can consider incorporating this information during consultations for multiple-gestation pregnancies occurring as a result of ART.

ART AND GENETICS

Reporting on the downstream effects of ART on neonatal genetic disease has historically focused on imprinting disorders, while the risks of ART association with other genetic conditions affecting neonates have not been comprehensively evaluated. A retrospective cohort study that compared the percentage of neonates conceived with or without ART vs the likelihood of finding a genetic diagnosis for their

symptoms discovered the diagnostic yield between ART-conceived pregnancies was comparable to that of naturally conceived neonates (10.1% vs 13.2%; OR, 0.74 [95% CI, 0.53–1.02]).[8] The incidence of de novo variants was also similar between ART- and naturally conceived neonates (75.9% [n = 41/54] vs 64.4% [n = 112/174]; OR, 0.89 [95% CI, 0.62–1.30]).[8] Wang et al also evaluated risks associated with ART and germline de novo variants.[9] The authors found that offspring conceived through ART had significantly more de novo variants than those conceived naturally, even after adjusting for parental age at conception.[9] The underlying cause of parental infertility was associated with an increased risk of de novo variants, and most of the de novo variants were of paternal origin, suggesting that the ART procedures themselves may not have been the cause of the increased frequency of de novo variants.[9]

From a chromosomal pT techniques on aneuploidy rates is an area of ongoing investigation. Kochanski et al reported increased frequency of sex chromosome aneuploidy; however, the authors do not account for the possibility of parental sex chromosome abnormalities.[10] Their study highlighted that ART can enable propagation of existing mutations. For example, ART may aid a male with a microdeletion in the AZF region with associated oligozoospermia to conceive, yet all male offspring would be expected to inherit the deleterious microdeletion.[10] From a culture medium perspective, the impact of embryo culture and other laboratory techniques on aneuploidy lacks concrete evidence.[11] In a study evaluating karyotypes of products of conception, abnormal karyotypes did not correlate to the method of conception (ie, ART vs naturally conceived pregnancies).[12]

When discussing ART and genetics, the option of preimplantation genetic testing for aneuploidy (PGT-A), monogenic disorders (PGT-M), and structural rearrangements (PGT-SR) must be considered. PGT is performed by obtaining a biopsy of a small sample of cells from the trophectoderm of a blastocyst and testing this sample for either chromosomal abnormalities or single-gene conditions. Testing methodologies vary based on the laboratory, but in general, next-generation sequencing and/or single-nucleotide variant analysis are used to detect chromosomal abnormalities; additionally, linkage analysis and/or direct detection are used to screen for single-gene conditions. The utility of PGT for polygenic risk scores and PGT for whole-exome sequencing is still being investigated, and they are not commonly used in IVF clinics currently. Patients undergoing IVF can screen for chromosomal abnormalities before a transfer by using PGT-A. This allows patients at higher risk of aneuploidy (eg, patients with Turner syndrome or advanced oocyte age) to reduce risk of aneuploidy in their offspring. PGT-A also allows any person considering IVF to reduce their risks of an aneuploid pregnancy and the possible associated birth defects or genetic syndromes. Patients at risk of passing on single-gene disorders or chromosomal conditions can elect PGT-M or PGT-SR to reduce the risk of passing a genetic condition to their offspring. In these ways, ART has a protective effect against the risk of offspring inheriting certain genetic conditions.[13,14] A Swedish study comparing outcomes and childhood health of pregnancies conceived via IVF with PGT vs IVF without PGT showed no significant increased risk of adverse effects from PGT, indicating the biopsy of the trophectoderm performed during PGT does not affect outcomes. Primary outcomes assessed were preterm birth and low birth weight. Secondary outcomes assessed were for early childhood health including asthma, allergic disorders, sepsis, hypothyroidism, attention deficit/hyperactivity disorder, autism spectrum disorders, affective disorders, schizophrenia, intellectual disabilities, cerebral palsy, epilepsy, and mortality from birth through end of follow-up in the study.[15]

Genetic counseling can be challenging due to the complexity of hereditary risk factors pursuant to IVF. Patients should be informed of the increased risk for imprinting disorders when ART is used; however, it should also be made clear that this risk is still much lower than 1%. Although the use of ART is ethically complex, counselors and providers should appropriately inform their patients on possible risks associated with ART while respecting their right to reproductive autonomy.[16]

ART AND IMPRINTING DISORDERS

Much of the discussion regarding the effects of ART on genetics and genetic disorders has revolved around potential increased risks of imprinting disorders in children conceived via IVF. The timing of ART manipulations, such as ovarian stimulation, intracytoplasmic sperm injection (ICSI), and embryo culture, overlap with the timing of epigenetic changes during the critical periods of fertilization and implantation. During this period, changes in DNA methylation levels are occurring, and the expression and impact of imprinted genes, particularly in more common imprinting disorders (Beckwith-Wiedemann syndrome [BWS], Russell-Silver syndrome [RSS], Angelman syndrome [AS], and Prader-Willi syndrome [PWS]), have been investigated in the setting of ART.[17,18]

A systematic review of DNA methylation levels and imprinting disorders in children conceived via ART concluded that children conceived via IVF, or ICSI, appear to be at a higher risk of any imprinting disorder than children

conceived naturally (odds ratio [OR], 3.67 [95% CI, 1.39–9.74]).[19] However, no significant association of altered DNA methylation in other individual imprinted genes was reported.[19] Furthermore, recent evidence on specific imprinting disorders suggests that BWS after ART is particularly elevated compared with other imprinting disorders such as RSS, AS, and PWS (relative risk, 5.2 [95% CI, 1.6–7.4]).[20] While this review did not find a statistically significant correlation for RSS, AS, or PWS, the authors attributed overall lower incidence rates of these conditions as a potential limitation. They also reported that while BWS did have higher prevalence in ART-conceived pregnancies, the study did not correct for fertility problems among the parents.[20]

In a 2007 analysis conducted by Doornbos et al that corrected for fertility problems among parents of children affected by BWS, AS, or PWS vs controls, the relative risk was the same, suggesting subfertility could play a role in the increased incidence of imprinting disorders in ART-conceived pregnancies.[21]

The investigation by Johnson et al[22] of the association between BWS and ART-conceived pregnancies focused on evaluating pregnancies with omphalocele and positive prenatal testing for BWS compared with those with omphalocele and negative BWS testing and found a 20-fold (P <.001) overrepresentation of positive BWS cases conceived via IVF compared with the rate of ART pregnancies in the United States. However, this study was likely confounded by ascertainment bias, given their population focused on pregnancies affected with omphalocele, which is a prenatal ultrasonogram finding known to be associated with BWS; thus, prenatal centers may have been more likely to request BWS testing in the setting of an IVF pregnancy.[22] Hattori et al[23] conducted a nationwide epidemiological study in Japan evaluating the association of ART with 4 common imprinting disorders (BWS, RSS, AS, and PWS). The authors found a 4.46-fold increased risk of BWS, 8.91-fold increase of RSS, 3.44-fold increase of PWS, and no statistically significant risk increase for AS (1.32-fold). Unique methylation patterns were observed in patients with RSS, leading the authors to conclude a distinct period of vulnerability to epigenetic changes exists immediately after fertilization, which is further exacerbated by techniques such as IVF, ICSI, and culture mediums, ultimately contributing to imprinting disorders. The inheritance pattern of BWS and AS are genetically heterogeneous. They can be caused by other inheritance patterns aside from imprinting defects, such as sequence variants, deletions, and uniparental disomy. Reviews have concluded BWS and AS conceived via IVF are more frequently associated with imprinting defects as the underlying genetic etiology when compared with children with BWS and AS who were conceived naturally.[24,25] Aforementioned, limited evidence also suggests ICSI may contribute to epigenetic errors and imprinting disorders. It is theorized that ICSI limits the ability for natural selection mechanisms because embryos are subject to environmental stressors that could lead to epigenetic changes, such as imprinting defects.[24,26]

In conclusion, data on the relationship between imprinting disorders and ART are varied but overall tend to support an increased risk of imprinting disorders, particularly BWS, in pregnancies conceived with ART vs those conceived naturally. It is important to note that causality cannot be inferred at this time, as the current literature does not account for imprinting risk that may come with subfertility, parental age, and other factors. Additional studies should be completed to further characterize this risk.

ART AND BIRTH DEFECTS

Multiple conflicting studies exist on the association between ART and birth defects. A study conducted in Western Australia, using registry data from 1993–1997, found that infants conceived with ICSI or IVF had twice the risk of major birth defects compared with infants born from nonassisted conception, including IVF, IVF/ICSI, ovulation induction, and intrauterine insemination.[27] However, an analysis of the South Australian Birth Defects Registry found no significant increase in major birth defects in IVF pregnancies after adjusting for parental characteristics, although a significant difference was observed in IVF/ICSI pregnancies.[28] An analysis conducted in Massachusetts compared singletons and multiple-gestation livebirths with respect to association of nonchromosomal birth defects in ART (ART-exposed), subfertile (ART-unexposed), and fertile (ART-unexposed) populations.[29] A higher prevalence of nonchromosomal birth defects was noted in ART births (199.1 per 10 000) compared with fertile births (139.9 per 10 000). The adjusted prevalence ratio (PR) for ART-conceived infants was 1.5 (95% CI, 1.3–1.6). On the other hand, 6183 of the 445 080 neonates born to fertile mothers had birth defects, with a prevalence rate of 138.9 per 10 000 live births. After adjusting for age, the PR of birth defects for ART-conceived infants was 1.4 (95% CI, 1.3–1.6). No significant difference was seen when comparing the age-adjusted PRs for mothers exposed to ART with mothers who have subfertility (PR, 1.2 [95% CI, 1.1–1.4]) (Table 3).[29]

A large US cohort study (2004–2015) using data from the SART Clinical Online Reporting System linked to state birth defect registries found that singletons conceived via IVF with or without ICSI had an increased risk of major congenital malformations, with male-factor infertility further exacerbating risk, compared with children conceived without

TABLE 3. Prevalence Rates and Age-Adjusted PRs for Nonchromosomal Birth Defects by Maternal Fertility Exposure Status

Birth defect	Prevalence Rate per 10 000 Live Births			Subfertile Prevalence Ratio[b] (95% CI)	ART[a] Exposed Prevalence Ratio[b] (95% CI)
	ART[a] Exposed (n = 17 829)	Subfertile (n = 9431)	Fertile (n = 445 080)		
Overall [c,d]	199.1	171.8	138.9	1.2 (1.1–1.4)	1.4 (1.3–1.6)
Cardiac Defect[d]	63.9	47.7	38.6	1.2 (0.9–1.6)	1.6 (1.3–1.9)
Conotruncal/Aortic arch	12.9	6.4	6.6	1.0 (0.4–2.2)	1.9 (1.3–3.0)
Tetralogy of Fallot	9.5		3.0		3.2 (1.9–5.5)
Left-Sided obstruction	6.2		6.2		0.9 (0.5–1.7)
Right-Sided obstruction	9.5	9.5	6.9	1.3 (0.7–2.9)	1.3 (0.8–2.1)
Pulmonary valve stenosis	8.4	8.5	5.6	1.4 (0.7–2.9)	1.4 (0.8–2.4)
Septal heart defects	35.9	26.5	20.5	1.2 (0.8–1.8)	1.7 (1.3–2.2)
Atrial septal defect[e]	24.1	14.8	14.0	1.0 (0.6–1.7)	1.6 (1.2–2.2)
Ventricular septal defect[f]	14.6	11.7	8.0	1.4 (0.8–2.6)	1.8 (1.2–2.7)
Non-cardiac Defect	145.8	133.6	109.1	1.2 (1.0–1.5)	1.4 (1.2–1.5)
Central nervous system	11.2		12.0		1.0 (0.6–1.6)
Respiratory defects	6.2		3.3		1.8 (1.0–3.4)
Orofacial	17.4	25.4	15.7	1.6 (1.1–2.4)	1.1 (0.8–1.6)
Cleft lip with/without cleft palate	10.1	9.5	7.0	1.4 (0.7–2.7)	1.4 (0.9–2.4)
Gastrointestinal	20.8	17.0	14.1	1.2 (0.7–2.0)	1.5 (1.1–2.1)
Genitourinary	47.1	44.5	32.9	1.3 (0.9–1.8)	1.3 (1.1–1.7)
Hypospadias, 2nd or 3rd degree[g]	45.8	25.1	23.8	1.0 (0.6–1.8)	1.8 (1.3–2.5)
Obstructive genitourinary defect	15.7	14.8	12.0	1.2 (0.7–2.1)	1.3 (0.9–1.9)
Musculoskeletal	54.4	40.3	41.1	1.0 (0.8–1.4)	1.4 (1.2–1.8)
Club foot	14.0	9.5	11.5	0.9 (0.5–1.8)	1.4 (0.9–2.1)
Craniosynostosis	7.9	7.4	4.0	1.8 (0.8–3.7)	1.9 (1.1–3.3)
Polydactyly/Syndactyly	14.0	19.1	12.9	1.6 (1.0–2.5)	1.2 (0.8–1.8)

Abbreviations: ART, assisted reproductive technology.
Prevalence rates reported per 10,000 live births. Prevalence estimates not calculated for case counts less than 6.
[a]Assisted reproductive technology.
[b]Adjusted for maternal age (<35 years, ≥35 years). Fertile group is referent.
[c]Any defect collected as part of surveillance by the Massachusetts Birth Defects Monitoring Program as described in Supplemental Table 1 [29]. Excludes cases with a Mendelian gene syndrome or chromosomal defect.
[d]Excludes patent ductus arteriosus.
[e]Does not include primum type.
[f]Does not include canal type or isolated muscular type.
[g]Prevalence per 10,000 male live births.
Printed with permission from Liberman RF, Getz KD, Heinke D, et al. Assisted reproductive technology and birth defects: effects of subfertility and multiple births. Birth Defects Res. 2017;109(14):1144–1153.[29]

ART (non-IVF/ICSI: aOR, 1.18 [95% CI, 1.05–1.32]; IVF/ICSI without male-factor infertility: aOR, 1.30 [95% CI, 1.16–1.45]; IVF/ICSI with male-factor infertility: aOR, 1.42 [95% CI, 1.28–1.57]).[30] Non-IVF/ICSI–conceived singletons were also at higher risk for cardiovascular defects (aOR, 1.2 [95% CI, 1.03–1.40]).[30] Additionally, when comparing ART-conceived singleton births, the use of ICSI was associated with increased risk of major nonchromosomal birth defects (aOR, 1.18 [95% CI, 1.03–1.35]).[30]

In summary, most studies indicate an increased risk of birth defects with IVF/ICSI, although the overall risk remains low (approximately 3.6% vs 3% in the general population). The Society of Maternal Fetal Medicine recommends that a fetal echocardiogram can be considered for pregnancies conceived via IVF with ICSI to allow for early detection of congenital malformations (Grade 2C, weak recommendation/low-quality evidence).[31]

ART AND STILLBIRTH

Current literature shows an increased risk of perinatal death in ART pregnancies. A systematic review and meta-analysis found a higher risk of stillbirths (defined as intrauterine death occurring at or after 20 weeks' gestation) in ART singleton pregnancies (OR, 1.82 [95% CI, 1.37–2.42]) compared with natural conceptions.[32] One study found a significantly greater risk of stillbirth in ART singletons born before 28 weeks' gestation, although no difference was found after 28 weeks' gestation.[33] Interestingly, ART twins had a lower stillbirth risk compared with naturally conceived twins until 40 weeks' gestation.[33] In comparison with pregnancies conceived with ART and/or infertility treatment (eg, ovulation induction, artificial insemination, or intrauterine insemination), the lowest stillbirth rate was seen at 38 weeks' gestation with progressively increasing stillbirth risk seen for infants

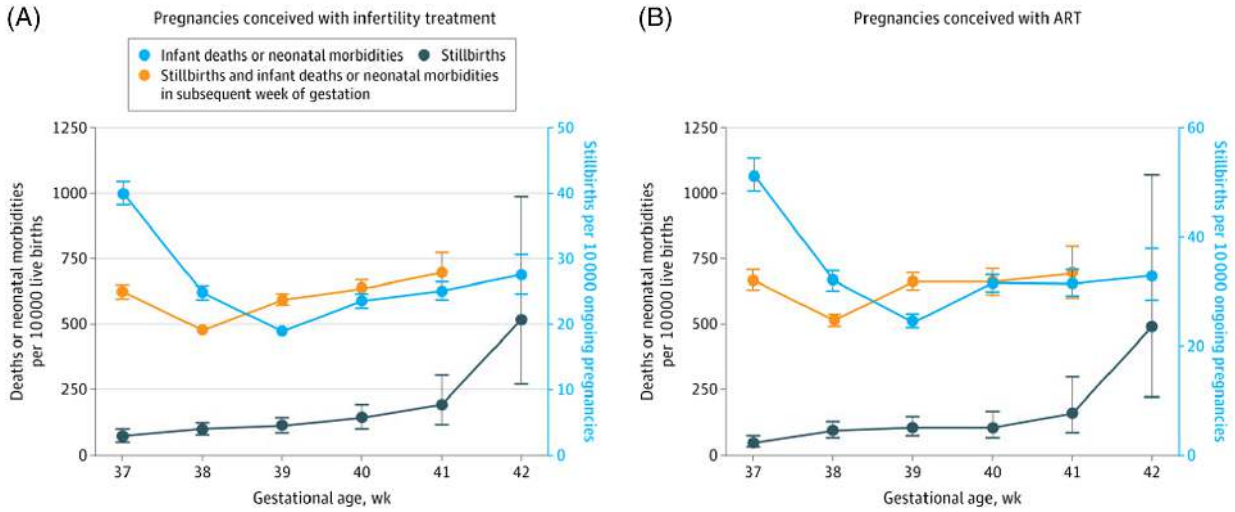


FIGURE 1. Comparison of rate of infant deaths or neonatal morbidities vs rate of stillbirths and infant deaths or neonatal morbidities in the subsequent week of gestation in term pregnancies conceived with infertility treatment or assisted reproductive technology (ART).[34]

born after 38 weeks' gestation (Figure 1).[34] Investigations into the association of stillbirth and ART are limited by the overall low incidence of stillbirths resulting in a smaller sample size and lower statistical power. Further research is needed to determine causal factors and ascertain delivery timing for ART-conceived pregnancies.

ART AND PERIPARTUM MORBIDITY

Pregnancies conceived via ART are at higher risk for abnormal placentation (placenta accreta spectrum, placenta previa, or vasa previa).[35–37] Studies linking these associations are confounded by type of embryo transfer, with frozen-embryo transfers associated with higher risk of anatomic, inflammatory, and vascular malperfusion abnormalities within the placenta.[38,39] The MOSART study found that abnormal placentation was among the strongest contributors to preterm birth in ART treatment.[40] ART singleton pregnancies also have higher cesarean section rates, with a 1.9-fold increase compared with natural conceptions. This includes a 1.91-fold increase in elective and a 1.38-fold increase in emergent cesarean section.[41,42] Postpartum hemorrhage and blood loss are also higher in IVF/ICSI pregnancies.[43] Early intervention, such as use of uterotonics at 500 mL of estimated blood loss, may help reduce these risks.

ART AND NEONATAL INTENSIVE CARE UNIT ADMISSION

ART is associated with preterm birth and increased neonatal intensive care unit (NICU) admissions. Current data are conflicting with respect to association of ART conception with greater need of respiratory support during NICU admission. Wang et al found that late-preterm infants conceived via ART had a higher risk of NICU admission (OR, 2.08 [95% CI, 1.1–4.7]) and required more aggressive respiratory support (OR, 2.53 [95% CI, 1.12–5.70]) compared with natural conceptions.[44] The study stratified ART conceptions into IVF and non-IVF fertility treatments. It demonstrated that noninvasive fertility treatments with pharmacologic agents such as selective estrogen receptor modulators, aromatase inhibitors, and gonadotropins have an increased risk of NICU admission (OR, 3.70 [95% CI, 1.3–10.7]) compared with nonassisted conceptions.[44] Furthermore, upon NICU admission, infants conceived with fertility treatments are more likely to need supplemental respiratory support, including continuous positive airway pressure, intermittent mechanical ventilation, or surfactant replacement therapy, compared with naturally conceived infants. Al-Hathlol et al found no detectable relationship with neonatal outcomes and duration of NICU stay among very low-birth-weight preterm infants conceived by IVF in comparison with neonates of unassisted conception.[45] As data on ART and NICU admissions are limited, continued research is needed in this area.

ART AND LONG-TERM OUTCOMES

Concerns about adverse cardiometabolic outcomes in children conceived via ART have been raised[46–48], although large cohort studies show no significant differences in cardiovascular disease (adjusted hazard ratio [aHR], 1.02 [95% CI, 0.86–1.22]) or type 2 diabetes (aHR, 1.31 [95% CI, 0.82–2.09]) between ART- and naturally conceived children.[49] A small increase in obesity has been associated with

ART conception (aHR, 1.14 [95% CI, 1.06–1.23]).[12] There has been an increased risk of type 1 diabetes seen in ART-conceived pregnancies from frozen-embryo transfer compared with fresh-embryo transfers (aHR, 1.52 [95% CI, 1.08–2.14]) and frozen-embryo transfers compared with natural conception (aHR, 1.41 [95% CI, 1.05–1.89]).[50]

Previously, the risk of cerebral palsy was found to be higher among pregnancies conceived through ART than natural conception (aOR, 2.76 [95% CI, 2.03–3.67]).[14] However, recent studies have shown that the risk has decreased (aOR, 1.39 [95% CI, 1.01–1.87]) due to the decrease in ART multiples and has been shown to be no longer associated with ART singleton pregnancies (aOR, 1.11 [95% CI, 0.98–1.24]).[51] Several systematic reviews have found no differences on language development, behavior, or social functioning for ART-conceived pregnancies.[52–54] Furthermore, educational outcomes and age-related development were equivalent between groups.[54]

The long-term impact on mental health for children born from ART must be considered as well. Previously, there was concern that children born from ART would have a higher prevalence of psychological disorders. Recent studies of children born from ART show no major psychological differences compared with naturally conceived children.[55,56] Specifically in the examination of reproductive donation, comparisons of families formed through donor gametes or gestational carriers vs families formed through natural conception showed no difference in adjustment problems, self-esteem, or psychological well-being by age 14 years.[57] In contrast, another study from Sweden showed a lower incidence of emotional and behavioral problems in children born to families who conceived using donor sperm or egg.[56] While the evidence above is reassuring, further longitudinal studies are warranted to understand the long-term impact beyond adolescence.

ETHICAL CONSIDERATIONS AND CHALLENGES

ART raises ethical concerns due to the inherent risks of fertility treatment, including venous thromboembolism, multiple gestation, preterm birth, and preeclampsia. These risks are further increased in patients with baseline comorbidities, such as an inherited thrombophilia, psychiatric disorders, and obesity.[58] ASRM advises reproductive endocrinologists to counsel patients on these risks and obtain consultations when needed. An example of this includes vascular Ehlers-Danlos syndrome, which carries greater risk of maternal mortality and a 50% chance of having an affected offspring. Providers should also discuss PGT-M and gestational carrier options for high-risk patients with complex genetic conditions.[58] Finally, as previously noted, ASRM promotes elective single-embryo transfer to minimize the risk of multiple gestations due to the associated increases in adverse obstetrical and perinatal outcomes of multiple gestations.[6] Shared decision-making and thorough counseling regarding risk are critical in these challenging ethical scenarios.

CONCLUSIONS

Over the past decade, ART guidelines have evolved to prioritize patient safety and optimize neonatal outcomes to adjust for rising use and success rates of ART. Further research is needed to assess the relationship between ART and adverse perinatal outcomes. The literature suggests ART may increase the risks of rare imprinting disorders in a pregnancy, but IVF with PGT can reduce the risk of many other more common genetic conditions. Recent improvements in accessing ART and perinatal outcome data will allow physicians to improve counseling of their patients on short-term and long-term risks associated with ART-conceived pregnancies for themselves and their future neonate.

American Board of Pediatrics Neonatal-Perinatal Content Specification

- Recognize abnormalities of the umbilical cord, fetal membranes, and placenta
- Understand pregnancy physiology

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NEO QUIZ

1. Infertility rates have increased in recent years. In the United States, data indicate that 1 in 5 women of reproductive age is unable to conceive within 12 months of trying. Infants conceived via assisted reproductive technology (ART) are at increased risk of preterm delivery and small-for-gestational-age status. According to a 2021 Centers for Disease Control and Prevention (CDC) report, what proportion of infants born in the United States are born via ART?

   A. 2%.
   B. 6%.
   C. 10%.
   D. 14%
   E. 18%.

2. Guidelines to decrease the risk of higher-order pregnancies after ART were published in 2021 by the American Society for Reproductive Medicine. While the incidence of higher-order pregnancies has declined, the incidence of twin gestations has remained high. What is the proportion of twin pregnancies due to non-in vitro fertilization (IVF) ART?

   A. 9%.
   B. 14%.
   C. 21%.
   D. 29%.
   E. 34%.

3. The risk of adverse perinatal outcomes is increased in IVF pregnancies with higher-order gestation compared with IVF singleton pregnancies. These include increased risk of preterm birth and low birth weight. Which of the following statements also represents an adverse perinatal outcome in higher-order IVF pregnancies compared with singleton IVF pregnancies?

   A. Increased risk of abruption.
   B. Increased risk of fetal growth restriction.
   C. Increased risk of gestational diabetes.
   D. Increased risk of pregnancy-induced hypertension.
   E. Increased risk of polyhydramnios.

4. In a systematic review, the risk of imprinting disorders in children conceived via ART was found to be increased. Which of the following imprinting disorder was recently found to be at a particularly increased risk?

   A. Russell-Silver syndrome.
   B. Angelman syndrome.
   C. Prader-Willi syndrome.
   D. Transient neonatal diabetes mellitus.
   E. Beckwith-Wiedemann syndrome.

5. While the risk remains low overall, studies indicate an increased risk of birth defects after IVF via intracytoplasmic sperm injection (ICSI). Which of the following intervention is recommended for pregnancies conceived via IVF/ICSI?

   A. Fetal brain magnetic resonance imaging (MRI).
   B. Fetal echocardiogram.
   C. Weekly nonstress test starting at 34 weeks of gestation.
   D. Early screening for gestational diabetes before 24 weeks' gestation.
   E. Increased folic acid intake to 4000 µg daily.

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Applications of Metabolomics and Lipidomics in the Neonatal Intensive Care Unit

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EDUCATIONAL GAPS

Clinicians caring for newborns should understand the translational relevance of the metabolome and lipidome and the potential for identification of novel mechanistic pathways and biomarker(s) for common disease outcomes such as bronchopulmonary dysplasia and necrotizing enterocolitis.

OBJECTIVES *After completing this article, readers should be able to:*

1. Describe commonly used nomenclature for terms related to evaluation and measurement of the metabolome and lipidome.
2. Recognize the potential impact of exposures on the neonatal metabolome and lipidome.
3. Characterize aspects of biomarker(s) identification for common neonatal and infantile disorders.
4. Describe technical and analytic considerations and challenges for metabolomic and lipidomic investigations.

ABBREVIATIONS

BPD	bronchopulmonary dysplasia
HIE	hypoxic-ischemic encephalopathy
IVH	intraventricular hemorrhage
LASSO	least absolute shrinkage and selection operator
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NEC	necrotizing enterocolitis
NMR	nuclear magnetic resonance
ROP	retinopathy of prematurity
SCFA	short chain fatty acids.

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ABSTRACT

The metabolome and lipidome comprise the thousands of molecular compounds in an organism. Molecular compounds consist of the upstream metabolic components of intracellular reactions or the byproducts of cellular pathways. Molecular and biochemical perturbations are associated with disorders in newborns and infants. The diagnosis of inborn errors of metabolism has relied on targeted metabolomics for several decades. Newer approaches offer the potential to identify novel biomarkers for common diseases of the newborn and infant. They may also elucidate novel predictive or diagnostic measures for a variety of health trajectories. Here, we review the relevance of the metabolome and lipidome for common disorders and highlight challenges and opportunities for future investigations.

INTRODUCTION

Newborns represent a vulnerable and understudied population because of organ underdevelopment and associated enzymatic and metabolic immaturity.[1] Molecular medicine and systems biology approaches including metabolomics and lipidomics represent an objective method to identify health and disease states.[2–5] Translating molecular medicine approaches by incorporating metabolomic and lipidomic data are critical for furthering biologic, clinical, and therapeutic insights. The metabolome is the comprehensive set of extra- and intracellular small-molecule (more than 1500 Da) substrates, intermediates, and end products (ie, amino acids, sugars, nucleotides, organic acids, lipids) that can be measured within a biological sample.[2,3,6] The metabolome provides a snapshot of the unique metabolic state of an individual patient. This may permit an understanding of both normal metabolic pathways and deviations that increase susceptibility to developmental and/or acquired disorders. The lipidome, a distinct field of its own, encompasses a chemically diverse cohort of nonpolar hydrocarbons that play a uniquely critical role in neonatal health.[7,8] Lipids are essential for a number of activities, including cell membrane formation, organogenesis, second messenger signaling, energy storage, and intercellular communication.[7–10]

Metabolomic and lipidomic profiling holds the potential to identify preclinical molecular alterations that signify susceptibility to disease. Many serious conditions that manifest in childhood, adolescence, and adulthood can be traced to metabolic abnormalities present in mothers and their offspring.[11] Early intervention by identification and detection of alterations in individualized metabolic and lipidomic data may significantly alter the course of these conditions, potentially reducing their clinical impact or mitigating their biologic effects. This principle serves as the underlying foundation of state-sponsored newborn screening programs nationally.[12] From a clinical perspective, comprehensive metabolomic and lipidomic profiling may facilitate the creation of targeted nutritional delivery. This is particularly beneficial in neonatal intensive care units (NICUs), where precise and personalized nutritional support can aid in growth, development, prevention of future disease, and repair from injury.

Fundamentally, investigations interrogating the neonatal metabolome and lipidome also advance general knowledge of developmental biology. Molecular biomarkers can provide benchmark and foundational developmental patterns for healthy newborns. They may also augment the utility of relatively nonspecific clinical definitions through incorporation of objective molecular markers or patterns of disease for outcomes and associated phenotypes that remain poorly defined. Through incorporation of objective molecular markers, researchers can subset nonspecific clinical definitions into more precisely defined metabolic categories. Most neonatal outcome definitions still rely heavily on nonspecific operational definitions that do not always precisely define pathobiology.[1]

This review will highlight general considerations, challenges, and opportunities for metabolomics and lipidomics as applied to neonates and infants. Definitions for techniques, approaches, and available databases are highlighted in Tables 1 and 2. We will provide a broad overview of metabolomics and lipidomics investigations in neonates and infants, outline considerations for the interested investigator and clinician alike, and highlight challenges and future opportunities for translational studies in the NICU. We refer interested readers to the many outstanding publications that cover preprocessing and analytic workflows, which will not be covered in this review.[5,11,13–19]

TECHNICAL CONSIDERATIONS

Metabolomics and lipidomics systematically identify and potentially quantify the diverse range of compounds present within a biological sample, providing a biochemical snapshot of the metabolic state of an organism (Figure 1A). Sample processing may involve various techniques, including nuclear magnetic resonance (NMR) spectroscopy and mass

TABLE 1. Definitions of Instruments and Terms Used in Metabolomics and Lipidomics Research

Instrument/Term	Definition	Characteristics
MS[a]	Measures the mass-to-charge ratio of ions, enabling molecular identification and quantification	Extremely sensitive and can detect compounds in very low concentrations Adaptable for both targeted and untargeted studies
Targeted MS[a]	Quantitatively analyzes a priori–selected metabolites or lipids	High precision and accuracy
Untargeted MS[a]	Identifies all present compounds without prior knowledge of what may be present. Comprehensive approach designed to capture a diverse array of compounds.	Often used for exploratory hypothesis-generating studies
NMR spectroscopy[a]	Applies a magnetic field and radiofrequency pulses to a sample to detect the energy changes of certain atomic nuclei (ie, ^{1}H or ^{13}C)	Unlike MS, does not require the ionization of samples and is nondestructive Highly reproducible and quantitative Generally less sensitive than MS and less commonly used in untargeted studies due to its lower sensitivity and the complex data interpretation it requires
LC-MS[a]	Combines the separation capabilities of LC with the analysis capabilities of spectrometry Used for analyzing complex biological samples containing biomolecules that vary in size and polarity such as in lipidomics studies Allows for separation of lipids prior to their ionization and detection, improving the specificity and sensitivity of the analysis	Adaptable for both targeted and untargeted approaches, providing extensive coverage and detailed structural insights
GC-MS[a]	Hybrid analytical method that is especially suited for the analysis of volatile and semi-volatile compounds Often used for the analysis of fatty acids, sterols, and organic acids The GC component separates compounds based on their volatility and interaction with the column material followed by their detection using MS	Incredibly sensitive and capable of detecting compounds at very low concentrations Requirement for compound volatility often necessitates derivatization of samples, which can complicate sample preparation
Annotated compounds	Compounds for which structural or functional information is already known and catalogued in available databases	Can be readily identified and quantified in biological samples using reference standards or by matching experimental data with existing data (ie, mass, retention time and spectral patterns) in comprehensive metabolite or lipid libraries
Unannotated compounds	Compounds that have not been previously identified or for which there is no available reference information in existing databases Identification of unannotated compounds generally follows a multistep process involving both advanced analytical techniques and computational tools	Represent a significant portion of the metabolites or lipids detected in typical untargeted analyses Pose both a challenge and an opportunity for researchers Identifying these unknown compounds is crucial for discovering new biomarkers or biochemical pathways but requires sophisticated techniques and approaches

Abbreviations: GC, gas chromatography; LC, liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance.
[a]The choice between these instruments and techniques largely depends on the specific objectives of the scientific question. For instance, LC-MS is preferred in lipidomics due to its ability to handle the diverse chemistry of lipid molecules, whereas GC-MS might be chosen for metabolomics involving volatile organic compounds. NMR, despite its lower sensitivity, provides valuable quantitative data for targeted analysis without derivatization or ionization, largely preserving the sample specimen. Each analytical technique used in targeted and untargeted metabolomics and lipidomics has strengths and weaknesses. MS provides high sensitivity and is versatile for both targeted and untargeted studies. NMR offers high reproducibility and structural information. LC-MS and GC-MS provide powerful combinations of separation and detection capabilities. The choice of method depends on the specific requirements of the study, including the nature of the sample, the types of metabolites or lipids of interest, and the desired level of molecular characterization.

spectrometry (MS), to analyze the complex chemical composition of cells, tissues, or biofluids. The processing of samples involves several steps to ensure accuracy and reproducibility. Initially, biological samples are collected and stored, often in −80 °C, to prevent degradation. Samples undergo preparation protocols that may include centrifugation, filtration, or derivatization, depending on the analytical technique to be used. The ultimate goal of these steps is to eliminate large molecules (ie, DNA, RNA, and proteins) while retaining hydrophobic and hydrophilic small-molecule metabolites and lipid compounds. High-throughput analytical platforms, primarily MS and NMR, are employed to detect and quantify metabolites and lipids. In MS-based metabolomics, samples are run through liquid or gas chromatography and ionized, allowing the mass-to-charge ratios of the metabolites to be measured via mass spectrometry. Fragmentation

TABLE 2. Databases That Provide Compound Matching and Identification[a]

Database	Description	Characteristics
MassBank of North America (MoNA) https://mona.fiehnlab.ucdavis.edu/	An extensive public repository of mass spectra for small molecules, including metabolites, contaminants, synthetic molecules, and biological macromolecules	It provides an open-access platform for researchers to search and match experimental mass spectra against a broad library of reference spectra
Human Metabolome Database (HMDB) https://hmdb.ca/	Contains detailed information about small molecule metabolites found in the human body Includes chemical, clinical, and molecular biology/biochemistry data	Specifically designed for human metabolic research Useful for linking metabolomic profiles to human health
METLIN https://metlin.scripps.edu/landing_page.php?pgcontent=mainPage	Metabolite database specifically developed for metabolomics by providing tandem mass spectrometry data for thousands of compounds across a variety of conditions	Often used for both identification and structural characterization of metabolites in biological samples
LipidMaps https://www.lipidmaps.org/	Categorizes lipids and provides detailed molecular information including mass spectral data, which is crucial for lipidomics studies	Focuses on lipids Provides comprehensive data on lipid structures and their biological functions
PubChem https://pubchem.ncbi.nlm.nih.gov/	Hosted by the National Center for Biotechnology Information A free database of chemical molecules and their activities against biological assays	Includes a vast amount of information on chemical structures, identifiers, chemical and physical properties, biological activities, patents, health, safety, toxicity data, and analytical spectra
ChemSpider https://www.chemspider.com/	Owned by the Royal Society of Chemistry Free chemical structure database provides access to over 63 million chemicals, properties, and associated information	Serves as a chemical search engine that aggregates and indexes chemical structures and their associated information into a single searchable repository
Golm Metabolome Database (GMD) http://gmd.mpimp-golm.mpg.de/	Database specializes in gas chromatography/mass spectrometry data	Particularly useful for metabolomics studies that involve GC-MS, providing reference spectra, retention indices, and metabolite profiles
National Institute of Standards and Technology Chemistry WebBook https://webbook.nist.gov/chemistry/	Provides a wide range of data on chemical and physical properties of pure compound	Particularly useful for matching and identifying compounds based on their mass spectra

Abbreviation: GC-MS, gas chromatography/mass spectrometry.
[a]Databases listed provide compound matching and identification. They provide extensive libraries of compound structures, spectra, and other relevant data that facilitate the identification of both known and unknown compounds. These databases are integral to the workflows in metabolomics and lipidomics, enabling researchers to identify unknown compounds, understand their functions, and link molecular profiles to biological pathways and disease states. Each database may specialize in different types of data or analytical approaches. Investigators will often utilize multiple databases to confirm compound identities and curate as much information as possible for a given molecule.

enables an additional dimension of identification, as metabolites have unique fragmentation patterns after collision with high-energy particles. The resulting data are processed through bioinformatics pipelines to identify and quantify (relative or absolute concentrations) the metabolites, typically involving peak detection, deconvolution, and normalization steps. The processed data are then analyzed using computational methods to interpret the metabolic signals and to identify significant changes related to the biological condition under study.[2,4,18,20]

Computational methods, including differential, multivariate, and machine learning techniques, identify significant differences and patterns in the high-dimensional biologic data (Figure 1B). Annotation and identification focus on matching molecular features to known databases or inferring structures of novel compounds. Univariate analyses are used to identify statistically significant associations followed by multiple hypothesis testing to control the false discovery rate. Pathway and network analyses map features to metabolic or signaling pathways and model interactions between individual molecules as well as between different pathways with distinct biologic function. Advanced machine learning methods including supervised and unsupervised approaches may be used for feature selection and predictive modeling. Validation and reproducibility techniques often employ training and test cohorts with cross-validation and external validation approaches. Thorough reporting and documentation of methodologies and workflows are crucial for transparency and reproducibility. These steps, while adaptable to specific requirements, form the foundation of transforming high-throughput biological data into meaningful insights.[2,21–23] The above approaches can be used for a variety of biological samples. Note, because of their chemical structures, metabolomic workflows interrogate mostly water-soluble polar compounds, whereas lipidomics focuses mostly on hydrophobic nonpolar compounds.[24] Although a wide range of chemical diversity exists among lipid classes, many individual compounds share structural similarities

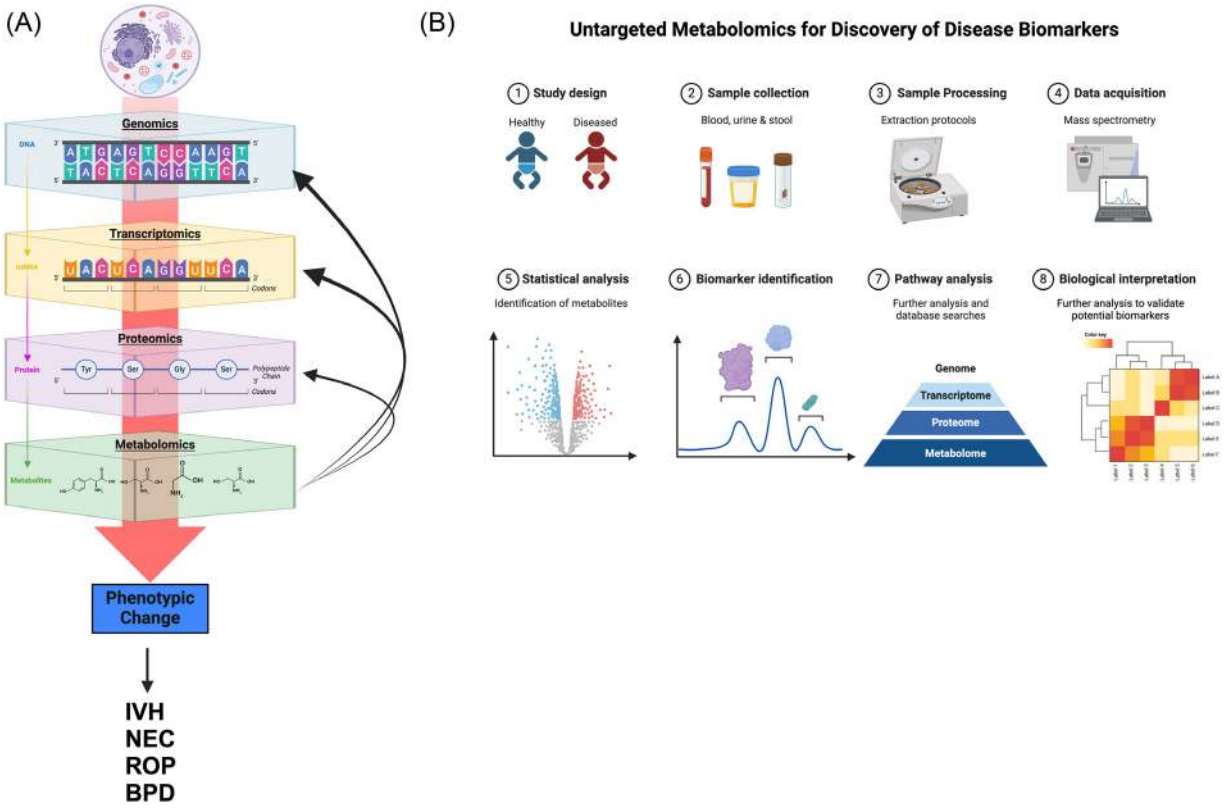


FIGURE 1. Overview of omics approaches and workflows. (A) The sequential layers of omics technologies, starting from genomics (DNA sequencing) to transcriptomics (messenger RNA analysis), proteomics (protein profiling), and metabolomics (metabolite identification). The arrows originating from metabolomics to the different layers signify feedback mechanisms, in which changes in metabolite levels can influence gene expression, protein activity, and messenger RNA level. (B) Workflow for untargeted metabolomics in the discovery of disease biomarkers for a hypothetical retrospective case-control study design. (1) **Study design** involves selecting healthy individuals and diseased patients. (2) **Sample collection** encompasses obtaining biological samples such as blood, stool, or urine. (3) **Pretreatment** includes extraction protocols to prepare the samples for analysis. (4) **Data acquisition** utilizes mass spectrometry to generate metabolic profiles. (5) **Statistical analysis** is performed to identify statistically significant metabolites. (6) **Biomarker identification** focuses on pinpointing potential biomarkers from the data. (7) **Pathway analysis** integrates further analysis and database searches to understand metabolic pathways. (8) **Biological interpretation** involves validating the potential biomarkers through additional studies, aiding in the comprehensive understanding of disease mechanisms and potential therapeutic targets. Figure created at BioRender.com.
Abbreviations: BPD, bronchopulmonary dysplasia; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity.

with permutations in double bond location or carbon chain length.[7]

Despite progress over the last 2 decades, challenges and limitations exist with current workflows. The complexity of the metabolome and lipidome and the vast diversity of metabolites require sophisticated analytical and computational tools, which can be resource intensive. Moreover, the sensitivity of compounds to preanalytical and analytical conditions necessitates rigorous standardization and quality control measures that are not always uniform across laboratories, core facilities, or institutions. The reproducibility and generalizability of study findings remain some of the greatest challenges of demonstrating widescale translational validity. This is compounded by cost and resource considerations that often result in relatively small study sizes. Despite these challenges, the continued advancements in analytical technologies and data analysis methods aim to progressively address these limitations.[25–28]

METABOLOMICS AND LIPIDOMICS DRIVE FUNDAMENTAL BIOLOGIC PROCESSES

Metabolite and lipid compounds play fundamental roles in human biology. They serve as major structural components of cellular membranes and heavily influence energy storage, signaling, and overall metabolic processes.[7] Owing to their biologic immaturity, preterm neonates often face challenges in achieving and maintaining a homeostatic balance of various compounds (ie, acylcarnitines, amino acids, free fatty acids, triglycerides) that are critical for their growth and development. This leaves them vulnerable to biochemical imbalances that can be measured.[29] For instance, essential

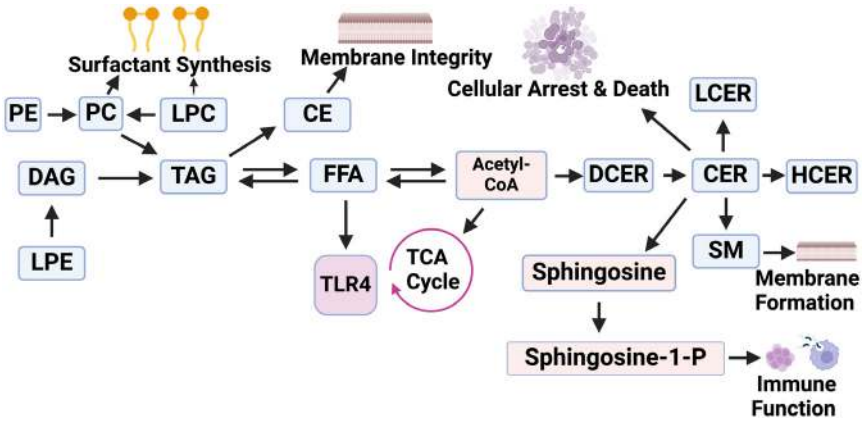


FIGURE 2. Lipid classes illustrating known relationships and mechanistic pathways. All lipid species are light blue. Metabolites are pink. Figure created at BioRender.com.
Abbreviations: CE, cholesterol esters; CER, ceramides; DAG, diacylglycerols; DCER, dihydroceramides; FFA, free fatty acids; HCER, hexosylceramides; LCER, lactosylceramides; LPC, lysophosphatidylcholines; LPE, lysophosphatidylethanolamines; PC, phosphatidylcholines; PE, phosphatidylethanolamines; SM, sphingomyelins; TAG, triacylglycerols; TCA, tricarboxylic acid.

and nonessential amino acids have diverse and critical metabolic functions including peptide and protein formation, nitric oxide production, and organogenesis. Branched chain (leucine, isoleucine, and valine) and aromatic amino acids (ie, phenylalanine, tyrosine, histidine, tryptophan) have highly conserved precursor roles in neurotransmitter production, protein folding, and organ development.[30]

Lipids, specifically phospholipids such as phosphatidylcholine and phosphatidylglycerol, are essential components of pulmonary surfactant.[31] Targeted lipidomics can assess phospholipid levels, enabling timely surfactant therapy to improve lung function and decrease morbidity and mortality.[32] Sphingolipids, including sphingomyelin, play key roles in cell membrane structure and immune signaling.[33] Abnormal sphingolipid metabolism is linked with respiratory distress syndrome and bronchopulmonary dysplasia (BPD).[34–36] Cholesterol is vital for membrane structure and synthesis of steroid hormones and bile acids, both essential processes for nervous system development.[37,38] Arachidonic acid and docosahexaenoic acid are critical for brain and retina development.[7,39] Note that many compounds have pleiotropic effects on various developmental processes and are not simply one-dimensional actors. For instance, eicosanoids, derived from arachidonic acid, mediate inflammatory responses significant in conditions such as BPD, retinopathy of prematurity, and periventricular leukomalacia.[7] However, under certain conditions, they are also growth factors important for cellular division and growth.[40] Triglycerides and free fatty acids are major energy sources essential for preterm infants' energy storage and cell-cell signaling.[41,42] They are also intricately linked with immune signaling, inflammation, and toll-like receptor 4 activation.[43] Thus, these compounds have specific and diverse roles in ongoing developmental biologic processes (Figure 2).

METABOLOMICS AND LIPIDOMICS: CLINICAL CONSIDERATIONS

Metabolomic and lipidomic studies include 2 key principles important for translational discovery: (1) determining novel biologic insights related to disease and (2) identifying molecular biomarkers associated with prognosis and established disease.[2] These principles are related but also distinct. The study of inborn errors of metabolism (IEMs) remains the best demonstrated application of metabolomics to date (detailed below). Beyond IEMs, most studies examining metabolomics and lipidomics for neonatal and infantile outcomes have considerable limitations, including small samples sizes, sampling from limited time points, and specimens obtained from a single biofluid. These issues have limited the overall reproducibility of findings.

Furthermore, certain factors are known to impact the metabolome and lipidome. Gestational age, weight, sex, nutritional status, and medications affect metabolite and lipid measurements.[22,29,44–47] However, findings are often compound dependent. For instance, many studies have demonstrated significant correlation between gestational age and metabolites including 3-hydroxyisobutyrate, acetate, acetoacetate, acetone, formate, glucose, and valine.[48,49] Lipid species such as free fatty acids, diacylglycerols, triglycerides, and others are all affected by age, weight, and nutritional considerations.[7,8] Accounting for these factors in clinical studies is necessary but often constrained by limited sample sizes and incomplete metadata. Thus, few studies to date have incorporated

adjustment, stratification, or the advanced epidemiologic or machine learning procedures necessary to isolate the biologic effect of a given metabolite level on a clinical outcome.[17]

The goal of most translational metabolomic and lipidomic studies is to characterize a clinical outcome or disease state using molecular patterns and/or biomarker(s). In newborn medicine, outside of IEMs, there are several use cases in which omics approaches have shown robust associations with neonatal disease.

Bronchopulmonary Dysplasia

Accumulating evidence demonstrates alterations in the metabolism of glucose, lipids, and amino acids in premature infants that develop BPD.[50] Premature infants exhibit high metabolic rates necessary for organogenesis and repair from injury.[51] BPD is defined principally by (1) alveolar arrest and (2) localized hyperimmune response to injurious exposures (ie, hypoxia and hyperoxia).[52] Numerous experimental and human-based omics studies suggest that abnormal metabolism of glucose and oxygen may lead to impaired cellular growth, apoptotic mechanisms, and abnormal repair mechanisms.[50,53] Recent studies demonstrate specific alterations in a range of species including sphingolipids, phosphatidylcholines, triglycerides, urea cycle amino acids (ie, arginine, citrulline), and others.[49,50,54,55] This growing evidence suggests that BPD may be characterized beyond traditional definitions to include the functional metabolic abnormalities that result from immaturity. While most studies to date have relied on sampling at a single time point to predict BPD occurrence at 36 weeks postmenstrual age, future studies should incorporate longitudinal metabolomic and lipidomic analyses for established BPD. Longitudinal sampling is critical to elucidating the multifactorial components that drive BPD pathobiology. Defining metabolomic and lipidomic measurements at the time of diagnosis is needed to define referent molecular signatures for established disease.

Necrotizing Enterocolitis

The prediction of medical and surgical NEC remains elusive for clinicians in the NICU. Diagnosis of medical NEC (ie, Bell stage II, limited mucosal injury NEC) has inherent subjectivity. Recent evidence suggests that NEC develops from a multifactorial process, including a dysbiotic microbial state.[56–59] Preterm dysbiosis, through gut colonization with *Enterobacter cloacae*, *Klebsiella pneumoniae*, and related taxa of the Gammaproteobacteria class, is most strongly associated with alterations in specific microbial metabolites found in stool including short-chain fatty acids, secondary bile acids, and additional markers.[56,60] These include free carnitine, short-chain acylcarnitines, long-chain acylcarnitines, urea cycle amino acids, sphingolipids, putragenic amines (putrescine), and the specific amino acids alanine and histidine.[60–64] As with most neonatal molecular studies, investigations of NEC to date have relied on sample cohorts from single centers while using a single time point for data analysis.[59] Results suggest the absence of a single molecular marker with sufficient sensitivity and specificity to inform clinical care; however, combinations of selected molecular features have illustrated reasonable predictive power for NEC (ie, area under the curve > 0.8).[59,61,63,65–70] The driving factors of NEC pathobiology remains unclear; it is unknown whether this process is driven by or simply associated with dysbiotic colonization patterns and microbial metabolite disturbances. Future studies would benefit from prospective longitudinal human sampling potentially incorporating multiple biofluid matrices (ie, blood, stool, urine) to fully characterize the microbial and metabolomic alterations associated with NEC onset and progression.

Hypoxic-Ischemic Encephalopathy

A limited number of studies have investigated the metabolome for infants with hypoxic-ischemic encephalopathy (HIE). Studies to date have demonstrated associations with 3-hydroxybutyrate, butyrate, various acylcarnitines (acetylcarnitine, palmitoylcarnitine, hexadecenoylcarnitine, stearolcarnitine), glycerol, succinate, *O*-phosphocholine, leucine, kynureine, and 3-hydroxydodecanoic acid.[71] Most studies have relied on serum umbilical cord blood or blood spots.[71,72] Future investigations may determine (1) metabolomic and lipidomic signals that correspond with HIE severity and (2) metabolomic patterns associated with response to therapeutic hypothermia.

Intraventricular Hemorrhage

The literature on neonatal intraventricular hemorrhage (IVH) indicates several metabolites and compounds associated with the condition. Amino acid alterations in alanine, arginine, asparagine, glutamine, lysine, valine, proline, taurine, and malate have been implicated in IVH compared to controls.[73] Organic acids such as lactate, malate, pyruvate, kynurenate, and hippuric acid were also found to differentiate between IVH and control cases.[73] Compared to other disorders related to prematurity, there is a relative paucity of omics studies that have examined IVH.

TARGETED METABOLOMICS FOR INBORN ERRORS OF METABOLISM

The realm of metabolomic and lipidomic studies for acquired neonatal conditions remains largely exploratory and hypothesis generating. However, newborn metabolic

screening for IEMs is an established and robust approach that unites biology and clinical actionability. The newborn screen is a critical public health program aimed at the early identification of IEMs by measuring molecular byproducts of defined metabolic pathways.

Early detection of IEMs prompts interventions intended to prevent the effects of the mutated metabolic pathway and consequent complications, including death. IEMs are typically monogenic conditions that lead to dysfunction in specific enzymes or proteins needed for endogenous metabolism.[12] Without proper treatment, this group of disorders leads to an accumulation of toxic substances or deficits in critical compounds, potentially resulting in acute toxicity, severe developmental issues, lifelong disability, or death. Conditions such as phenylketonuria, maple syrup urine disease, and medium-chain acyl-CoA dehydrogenase deficiency are among the few dozen diseases tested for in the standard newborn screening panel.

The methodology employed in screening for IEMs is based on targeted metabolomics. This approach focuses on measuring a few dozen metabolites from whole blood collected on a filter paper from a heel prick.[12] Newborn screening programs have revolutionized the early detection of inborn errors of metabolism through the application of targeted and sequential tandem mass spectrometry (MS/MS). The precision and specificity of this method make it exceptionally useful for detecting the subtle metabolic changes indicative of rare metabolic disorders.[12] The ability of MS/MS to detect a wide range of metabolic abnormalities from a minimal sample volume has made it an indispensable tool in newborn screening.[12] This application of MS is arguably the most impactful molecular screening measure in all of medicine on a population scale to screen for rare disorders, as it facilitates early diagnosis and intervention. Thus, the integration of MS/MS into newborn screening programs offers a powerful use case to safeguard the health of newborns.

NEWBORN SCREENING FOR COMPLEX PHENOTYPES

Although the newborn screen is primarily utilized in clinical practice to screen for IEMs, newer investigations have examined its potential for predicting complex phenotypes. We and other investigative groups have interrogated the newborn screen as a predictor for acquired disorders of prematurity and other multifactorial disorders. In many cases, this approach hypothesizes that a complex collection or pattern of metabolites may inform the prediction of a later outcome. Given that the newborn screen is a population screening tool with available linkages to maternal and neonatal clinical records, investigators have leveraged this data to evaluate thousands of patients rather than the few dozen or hundred(s) typical of many untargeted approaches. This approach also potentially overcomes reproducibility issues incumbent to many of the untargeted approaches historically used. Analyses often rely on complex regression and/or machine learning methodologies, interrogating acylcarnitines, and amino acids—the principle analytes on the newborn screen. Disorders evaluated include acquired disorders of prematurity—BPD, NEC, sepsis, and mortality.[63,74–77] They also include term diagnoses such as persistent pulmonary hypertension of the newborn and HIE.[72,78,79] There is also a growing recognition that the newborn screen may reflect common maternal conditions during pregnancy such as hypertensive disorders of pregnancy or diabetes.[75,79,80] For instance, infants born to mothers with hypertensive disorders of pregnancy have demonstrated alterations in short-chain acylcarnitines compared to controls.[77] Moreover, infants born to mothers with diabetes (any form) were found to have alterations in long-chain acylcarnitines, again compared to controls.[77] Early detection of amino acid and acylcarnitine abnormalities may reflect the molecular conditions necessary for inflammatory and/or oxidative injury to occur. Elevations in long-chain acylcarnitines are strongly associated with inflammatory pathways.[80] For diseases with overlapping pathobiology rooted in maladaptive responses such as NEC, BPD, and retinopathy of prematurity, these approaches have the potential to reveal individualized levels of risk across various populations.

TIMING AND LOCATION OF SAMPLE COLLECTION

The timing of sample collection in omics studies is crucial for accurately interpreting biochemical alterations in relation to a specific outcome. Samples collected prior to the onset of clinical sequelae may reflect underlying biochemical changes that predispose to disease. Samples obtained during active disease (ie, established BPD) can reveal ongoing metabolic disturbances that reflect disease activity. Analyses at different stages can distinguish between progressive, active, and established states. They can also aid in determining normal biologic variation over time—necessary when evaluating compounds that have never been characterized in the neonatal population. These temporal dynamics are also crucial for understanding metabolic and lipidomic trajectories between active and quiescent disease states while informing targeted therapeutic interventions and management strategies. Longitudinal analyses can also considerably increase statistical power when compared to cross-sectional studies.[81]

The choice of sampling location is also critical, as it directly influences the type of compounds detected, which in turn affect interpretation. Different sample types—blood, serum, plasma, tracheal aspirates, urine, stool, and tissue—each provide unique insights into the organism's metabolic and lipidomic status, reflecting various physiological and pathological states. Blood, serum, and plasma are among the most commonly used sample types, owing to their relative accessibility and the rich information they contain about systemic metabolism. These biofluids are particularly valuable for diagnosing and monitoring systemic diseases and for understanding overall metabolic homeostasis.[3,4,6,21] However, they may not reveal localized perturbations in metabolic processes for organ-specific processes. Urine and stool samples offer complementary dimensions, reflecting excretion processes. Urine studies can reveal information regarding filtering and excretion processes.[82] Stool samples can offer information on local microbial activity, immune regulation, and the influence of these factors on systemic health.[83] Tracheal aspirates can be useful for respiratory metabolism, lung diseases, or systemic conditions with pulmonary manifestations.[34,53,84–86] Tissue samples, including biopsies from specific organs or tumors, offer the most specific and localized information but are difficult to obtain, owing to the invasive methods required for collection. Interpreting results in the context of the distinct source is critical for appropriate biologic and translational interpretation.

TOWARD ACTIONABLE PHARMACOMETABOLOMICS

Pharmacometabolomics is an emerging field that combines the principles of pharmacology and metabolomics. This discipline focuses on studying metabolomic profiles that can predict or explain drug responses at an individual level. For neonates, developmental differences (ie, enzymatic maturation), genetic polymorphisms, epigenetic marks, and environmental factors create a unique constellation of factors that need to be considered when interpreting pharmacokinetic and pharmacodynamic data.[87] Traditional dosing strategies in neonates are often extrapolated from adult data, adjusted for body weight or surface area, which can lead to suboptimal dosing. Pharmacometabolomics can improve upon suboptimal dosing by identifying metabolic signature(s) that predict how a neonate may respond to a specific drug, leading to safer and more effective dosing. These approaches may also help in discerning patterns that predict safety or efficacy.

There have been a limited number of pharmacometabolomic investigations in neonates. Studies have examined inhaled or systemic steroids with sampling from blood or urine before and after medication administration. Lewis et al measured blood and urine metabolomic response to dexamethasone for preterm infants at risk for BPD.[88] Serum gluconic acid sharply decreased in response to dexamethasone administration, with a median fold change of 0.007 across time. In metabolite set enrichment analyses, ammonia recycling and malate-aspartate shuttle pathways were enriched when comparing before and after treatment. In a follow-up validation study, investigators observed changes in urine citrulline and serum trans-4-hydroxyproline levels that showed similar results to their original investigation.[89] Citrulline is a key urea cycle metabolite important for endogenous nitric oxide, providing a vasodilatory effect in peripheral and pulmonary vasculature. Trans-4-hydroxyproline has been previously associated with pulmonary fibrosis and and/or lung injury.[90] More recently, Ballard et al evaluated blood metabolomics in a pilot trial of budesonide in surfactant.[91] They observed 90 distinct metabolites increased or decreased in a time- and dose-dependent manner, with an overrepresentation in lipid and amino acid pathways. However, when comparing their results directly with Lewis et al, there were no significant changes for the majority of detectable metabolites. One metabolite, pantothenate, was significantly increased; however, Lewis et al reported a 4-fold decrease. Follow-up studies may consider further evaluating pantothenate for its association with systemic steroid administration. Finally, Torgeson et al compared the urine metabolome in infants receiving hydrocortisone as part of the of the Trial of Late Surfactant (TOLSURF).[92] Of the 1145 urinary metabolites detected, 245 metabolites significantly changed over time, with cortisol derivatives increasing 2-fold following hydrocortisone therapy.[92] The highlighted studies are key first steps in this relatively new field. Future studies must evaluate both established and novel therapies beyond steroids. Prime targets may include exogenous surfactant, caffeine, and antibiotics.

METABOLOMICS AND THE MICROBIOME

The relationship between metabolomics, beneficial commensal organisms, and alterations in the microbiome is an increasingly relevant area for newborn health.[56] Fundamental areas of interest include (1) understanding the developmental timing and establishment of the microbiome in the setting of varied environmental prenatal and postnatal exposures, (2) determining commensal vs pathogenic influence, and (3) defining dysbiosis in terms of its impact on endogenous and exogenous metabolites. Many data (although not all) suggest that the fetus resides in a

sterile in-utero environment.[56] Colonization occurs via passage through the birth canal during a vaginal delivery with initial gut colonization that includes *Streptococci*, *Lactobacilli*, and *Enterobacteriaceae*.[56] The subsequent development of a rich gut microbiota predominant in commensal *Bacteroides* and *Bifidobacteria* species and related organisms is linked with early changes in stool patterns, from sterile meconium to the soft semi-formed consistency typified after feeding is firmly established. Microbial metabolites such as short-chain fatty acids (SCFAs) and bile acids are directly linked to the fermentation patterns represented by the colonizing gut microbes and thus may serve as early molecular patterns mediating clinical changes.[93,94]

SCFAs are a group of short carbon chain (typically 2–6 carbons) metabolites (ie, acetate, propionate, butyrate) generated by gut microbial fermentation of complex carbohydrates.[93] SCFAs supply energy to colonocytes and regulate immune responses while maintaining the integrity of the gut barrier. Clinical outcomes are linked with the relative abundance of SFCA species. Recent work demonstrates changes in specific SCFAs associated with decreased risk for disorders such as BPD and NEC.[49,95] SCFAs are produced in part by *Bifidobacterium* species that metabolize human milk oligosaccharides from human milk. Interestingly, premature infants have decreased levels of commensal *Bifidobacterium* and *Bacteroides* organisms compared to term infants[96] and increased pathogenic bacteria including *Klebsiella*, *Enterobacteriaceae*, *Staphylococcus*, *Streptococcus*, and others. These colonization patterns reflect a multitude of factors representing those occurring secondarily to NICU exposures such as widespread perinatal antibiotic usage, prevalence of human breast milk feeding, minimal initial enteral feeding, and reliance on total parental nutrition. *Bifidobacterium* species play a crucial role in metabolizing prebiotic human milk oligosaccharides to produce an abundance of the SCFA acetate and lactate. These metabolites provide essential ecologic service to the newborn gut by lowering the gut pH, thus shaping the balance of colonizing commensals (strict anaerobes) and potential pathogens (microaerophilic microbes).[56,97] This relationship is critical for the establishment of a eubiotic (high in anaerobes fermenting human milk oligosaccharides) state while displacing dysbiotic microbes (facultative anaerobes, ie, gammaproteobacteria) that have been most closely associated with devastating diseases such as NEC.[97,98] A recent U.S. Food and Drug Administration letter published in September 2023 to halt the clinical administration of *Bifidobacterium* and other multi-strain probiotics has currently limited clinical and translational work in this area.[99]

Besides SCFAs, secondary bile acids are additional microbial metabolites produced in the gut through the action of gut microbiota on primary bile acids.[96] Primary bile acids, synthesized in the liver from cholesterol, are secreted into the intestine, where they are modified by gut bacteria, particularly by *Clostridium*, *Bacteroides*, and *Bifidobacterium* species, into secondary bile acids such as deoxycholic acid and lithocholic acid.[93] The production of secondary bile acids also affects gut ecology, resulting in homeostatic control of systemic bilirubin and bile acid metabolism (eg, hyperbilirubinemia) as well as also shaping the ecology of the gut-colonizing microbes.[93] Disruption in these tightly controlled processes can cause a buildup of cytotoxic levels of hydrophobic bile acids within enterocytes and may lead to NEC. Thus, secondary bile acids play complex roles in host metabolism and health. While they aid in the digestion and absorption of dietary fats and fat-soluble vitamins, perturbations have been linked to clinical sequelae including NEC and brain injury.[100–103] The intersection of metabolomics and the microbiome offers tremendous potential in advancing our understanding of neonatal health and disease. In analyzing how microbial communities alter host metabolites and how these metabolites in turn affect microbial composition, there may be the ability to therapeutically manipulate these interactions in favor of anti-inflammatory and pro-growth trajectories.

COMPUTATIONAL AND MACHINE LEARNING APPROACHES

The complexity of metabolomic and lipidomic data often necessitates the use of advanced computational methods for analysis. Generally, bioinformatic pipelines are used to process the data into intensity values for various metabolites and lipids (both annotated and unknown). This tabular data (in which rows represent samples and columns represent intensity values for various species) is optimal for analysis by standard machine learning approaches. In supervised machine learning, this data can be used alongside labeled outcome data (eg, annotations for each sample indicating if the newborn had an outcome of interest) to create predictive models. Unsupervised approaches (ie, those that do not require labeled outcomes) can be used to reduce the complexity of the data, removing noise and helping to visualize and cluster analytes into groups that may reflect biological meaning.

Owing to the high dimensionality of omics data, often with relatively small sample sizes, methods designed to analyze high-dimensional data tend to be the most suitable and offer the best performance. For example, least absolute

shrinkage and selection operator (LASSO) is a form of regularized regression, which introduces a penalty for each feature that is used in the final model.[104] The resulting models often use a relatively small number of analytes. An important aspect of supervised machine learning is to split the data into training and validation sets. The training set is used to develop the model, and the validation set is used to estimate its performance on unseen data. This process is generally repeated multiple times with different train-validation splits in a process called cross-validation.[105] Models trained via regularized supervised machine learning approaches such as LASSO often generalize better to unseen data. Furthermore, by computing feature importance (ie, a measure of the contribution of each input variable to the model's prediction), these models can lead to hypotheses for novel biomarkers.

More advanced computational methods are also being applied to neonatal metabolomics and lipidomics research. Deep learning approaches, such as convolutional neural networks, are currently under exploration for their ability to capture complex, nonlinear relationships in high-dimensional data in maternal and child health. For example, De Francesco et al used a multitask deep learning model to predict 24 neonatal outcomes incorporating longitudinal maternal and neonatal data.[106] Shen et al used convolutional neural networks to analyze pseudo-images that represented metabolomics data, showing an improved ability to predict gestational age compared to traditional bioinformatic pipelines.[107] Deep learning approaches also enable the integration of heterogeneous data types, allowing the simultaneous analysis of metabolomics, lipidomics, clinical data, and other data modalities.[108] Furthermore, deep learning approaches are often flexible with respect to the ways in which they combine data modalities. Data can be combined before machine learning modeling and used as inputs to train a single machine learning algorithm called early fusion. Individual models can also be trained on each modality separately and combined using a meta-learner (which learns the best way to combine predictions from each individual model); this approach is called late fusion (sometimes called stack generalization).[109] As these computational approaches evolve, they promise to help enhance our understanding of neonatal biology and ultimately improve clinical care.

BIOLOGIC SAMPLING IN CLINICAL TRIALS

Most human-based metabolomic and lipidomic investigations rely on retrospective case-control study designs. To advance translational medicine, there is a critical need to incorporate biologic sampling within clinical trials. The underlying mechanisms for established therapeutic interventions and pharmacotherapies in humans remain unknown in up to 25% of cases.[110] The evolution of clinical trials beyond the limitations of clinical outcome data alone is important for furthering biologic and clinical insights.

Cost considerations often dictate the ability to pursue concurrent biologic sampling. However, recent illustrative examples highlight the potential for examining both biologic and clinical endpoints.[111,112] In the Bilirubin Neurotoxicity and Neurodevelopmental Impairment in Extremely Preterm Infants trial led by Dr Lindsay Holzapfel (Clinical Trials number NCT04584983), daily blood samples were obtained from extremely preterm infants to measure unbound bilirubin and unbound free fatty acid profiles from infants randomized to receive usual vs reduced dosing of lipid emulsions.[111] Clinical outcomes measured neurotoxicity through brainstem auditory evoked response testing. Small volume blood sampling measured free fatty acid profiles. The results of this study may shed light on the relationship between lipid emulsion dosing, free fatty acid profiles, and the presence or absence of neurotoxicity.

The High-Dose Erythropoietin for Asphyxia and Encephalopathy trial evaluated plasma inflammatory mediators in infants with moderate or severe HIE.[112] Notably, erythropoietin administration did not confer a protective effect. However, concurrent blood sampling enabled a secondary analysis of erythropoietin's effect on systemic inflammation and predictive performance for neurodevelopmental impairment. The study found that erythropoietin did not significantly alter concentrations of measured biomarkers by postnatal day 4. However, 6 plasma analytes were found to modestly increase the predictive power (area under the curve from 0.73, 95% CI, 0.70–0.75 to 0.79, 95% CI, 0.77–0.81) for death or neurodevelopment impairment at 2 years compared to clinical data alone. These results demonstrate feasibility and the potential utility for measuring biologic and clinical outcomes in clinical trials.

CONCLUSION

Metabolomic and lipidomic investigations have the potential to advance precision and individualized medicine approaches in the NICU. Untargeted and targeted approaches can characterize biochemical patterns associated with a variety of important clinical outcomes. In time, they may augment or even replace reliance on imprecise clinical definitions. Importantly, longitudinal sampling may characterize novel developmental trajectories and aid in identifying mechanistic and metabolic pathways that drive complex phenotypes of disease. A combination of traditional and

advanced machine learning methods can be used to account for high-dimensional omics data. Results must be interpreted in the context of sample location and timing of collection. Incorporation of clinical context data and the reliance on the knowledge and acumen of practicing providers is paramount. Ultimately, leveraging interdisciplinary expertise is critical to ensure the reproducibility and rigor of findings. Future studies may examine manipulation of key metabolic pathways to aid in prevention and/or treatment of common neonatal and infant diseases.

American Board of Pediatrics Neonatal-Perinatal Content Specification

- Demonstrate proper assessment of fetal well-being
- Understand technical considerations and data science opportunities in metabolomic and lipidomic studies in the neonatal intensive care unit
- Recognize the potential for novel mechanistic and biomarker development for common disorders in term and preterm neonates

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INDEX OF SUSPICION

Multicystic Lobar Lung Lesion in a Preterm Neonate

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CASE PRESENTATION

A female infant aged 24 weeks and 5 days gestation is born to a G3P1 mother aged 30 years old via cesarean section for breech presentation in the setting of preterm labor. The mother receives a course of antenatal steroids. Pregnancy is complicated by type 2 diabetes mellitus. The infant's birth weight is 581 g (22.5 percentile for weight on the Fenton growth chart), with proportional head circumference and length. The infant is intubated at delivery, receives surfactant, and stabilizes on first intention high-frequency oscillatory ventilation as per the center's protocol. The initial chest radiograph at birth is consistent with respiratory distress syndrome. Due to the increasing fraction of inspired oxygen (FiO_2) requirement to a maximum of 0.85, a second dose of surfactant is given on the day of life 4. She is transitioned to a high-frequency jet ventilator (HFJV) and receives a third dose of surfactant on the day of life 9, given poor oxygenation on FiO_2 of 1. Her oxygenation remains challenging, and a chest radiograph demonstrates optimal endotracheal tube position, multiple cystic lucencies in the right lower lobe with focal hyperinflation, and associated mass effect with right upper lung collapse and mediastinal shift. An 18-day course of intravenous dexamethasone is started on day 12 of life as the FiO_2 requirement remains high. She later develops a large, well-demarcated lucency in the anterior midline chest representing pneumomediastinum, so HFJV settings are adjusted. The 4 sigh breaths were removed, and the positive end-expiratory pressure decreased from 14 to 12 cm H_2O, with subsequent resolution of pneumomediastinum by the next day. Her oxygenation remains suboptimal with worsening cystic

ABBREVIATIONS

CLE	Congenital lobar emphysema
CPAM	Cystic pulmonary airway malformation
CT	Computed tomography
FiO2	Fraction of inspired oxygen
HFJV	High-frequency jet ventilator
NICU	Neonatal intensive care unit
PIE	Pulmonary interstitial emphysema
PMA	Postmenstrual age

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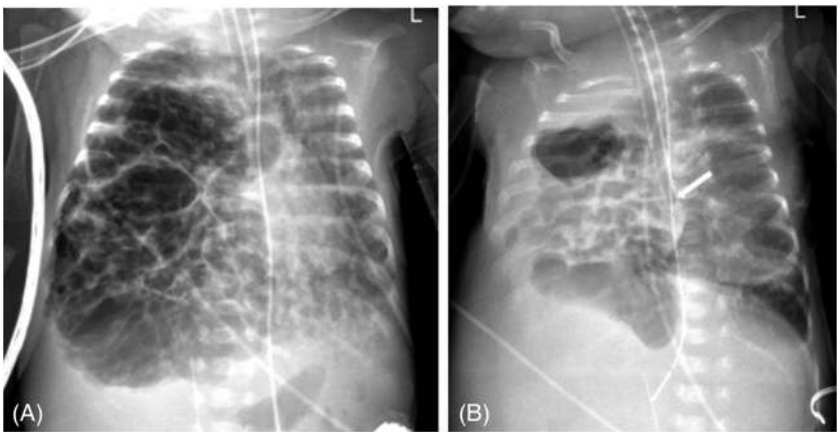


FIGURE 1. Left (A): Frontal view of the chest radiograph on day 27 of life demonstrating right lower lung cystic lucencies with worsening of focal lung hyperinflation and mass effect. Right (B): radiograph chest frontal view on day 33 of life, during left main bronchus intubation trial. The tip of the endotracheal tube is seen in the left main stem bronchus (white arrow). There is hyperinflation of the left lung with some residual scattered focal atelectasis. Right lung hyperinflation has decreased with a decrease in the size and number of multiple cystic lucencies.

changes with emphysematous blebs in her right lung (Figure 1A). The infant continued to be managed on HFJV over the next several days. Many interventions are attempted, including antibiotic treatment of possible pneumonia, repositioning, sedation, and paralysis, without sustained improvement. A trial of left mainstem intubation (Figure 1B) is pursued for a few hours, which she did not tolerate with ensuing severe hypercarbia and needing a FiO_2 of 1, prompting retraction of the endotracheal tube from the left main stem bronchus. As the infant's clinical condition is not improving on different ventilator strategies, including a trial of single-lung ventilation, general surgery is consulted for possible lobectomy. She is transferred to the level IV neonatal intensive care unit (NICU) on the day of life 36.

DISCUSSION

Differential Diagnosis

The differential diagnosis of cystic lung lesions in this premature infant includes severe cystic pulmonary interstitial emphysema (PIE) secondary to ventilator-associated lung injury, cystic pulmonary airway malformation (CPAM), and congenital lobar emphysema (CLE). Other differential diagnoses in an infant presenting with cystic lung disease include necrotizing pneumonia, pneumatocele, congenital diaphragmatic hernia, and cystic bronchopulmonary dysplasia.

Progression

After transferring to the level IV NICU for surgical evaluation, the patient becomes critically ill and hypotensive, likely due to mediastinal compression by the significant cystic changes in the right lung. After aggressive fluid resuscitation and inotropic support, a computed tomography (CT) of the chest with contrast is obtained, revealing a large right lower lobe multicystic lesion, which the radiologist reports to be consistent with a type I CPAM (Figure 2). The following day, at 30 weeks and 1 day postmenstrual age (PMA) and weighing 1300 g, she underwent a right lower lobe lobectomy with a preoperative diagnosis of type 1 CPAM. A surgical specimen is sent for pathology review. The patient rapidly improves postoperatively, and ventilator settings are weaned as tolerated. She receives a 10-day course of dexamethasone to aid with extubation. She is successfully extubated (Figure 3), weaned progressively to room air by 36 weeks PMA, and discharged home at 39 weeks PMA on room air, taking all oral feeds.

The patient is now 62 weeks PMA, thriving well, and is healthy without any respiratory issues.

Actual Diagnosis

Histopathology confirmed the diagnosis of cystic PIE (Figure 4).

The Condition

PIE is a ventilator-associated acquired disease of preterm neonates caused by air leakage into the interstitial space and characterized by radiolucency along the perivascular sheaths. PIE causes ventilation/perfusion mismatch and compression of unaffected lung tissue and can lead to pneumothorax, pneumomediastinum, and/or pneumopericardium.[1,2]

Managing PIE can be complex, as increased support from mechanical ventilation may worsen the cystic lesions, which may compress the unaffected adjacent lung tissue, impairing oxygenation and ventilation. Thus, it may be necessary to tolerate hypercapnia while attempting to wean the mean airway

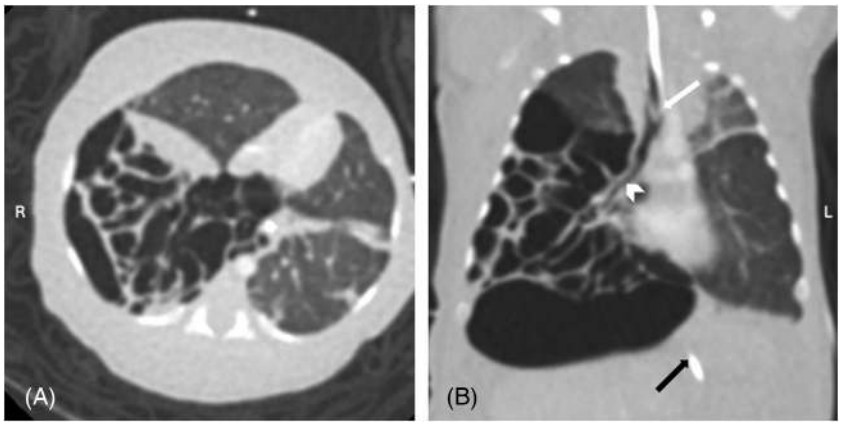


FIGURE 2. CT chest lung window axial (A) and coronal (B) images demonstrate multiple round, ovoid, and oblong-shaped cystic lucencies involving the right lower lobe having an appearance similar to the cystic mass of type I CPAM. In the coronal image, the right lower lobe bronchus is seen entering the hyperinflated multicystic lower lobe (arrowhead). The left lung is well expanded and shows some small focal atelectasis. The endotracheal tube tip is in the lower thoracic trachea (white arrow). A nasogastric tube is seen in the stomach (black arrow).

pressure to prevent worsened distension. Positioning the affected side of the chest down and using high-frequency jet ventilation help decrease further distension and worsening of PIE. For the subset of patients whose cysts worsen and coalesce, there may be a role for single-lung ventilation, as described by Rastogi et al.[3] In term infants, a trial of selective bronchial blockade can be attempted to determine if single-lung ventilation can be tolerated before subjecting the infant to lobectomy.[4] In refractory cases, there are reports of surgical intervention with lobectomy.[5,6] Histopathology provides a definitive diagnosis of the PIE, and resection may be curative in the most severe cases.

Based on this experience, we only had a definitive diagnosis after resection. To our knowledge, there is no literature in pediatric surgery regarding the optimal timing for surgical intervention in cystic lung lesions in premature infants. In the future, we recommend considering early surgical consultation and CT imaging to better delineate the extent of lung involvement. If severe diffuse cystic PIE involves a single lobe of the lung and is not responding to medical management, surgical resection of the affected lobe can be curative.

LESSONS FOR THE CLINICIAN

- While rare, refractory PIE may present as a worsening cystic lung disease that progresses to the point of hemodynamic instability.
- High-frequency ventilation and patient positioning are the first lines of management for PIE.

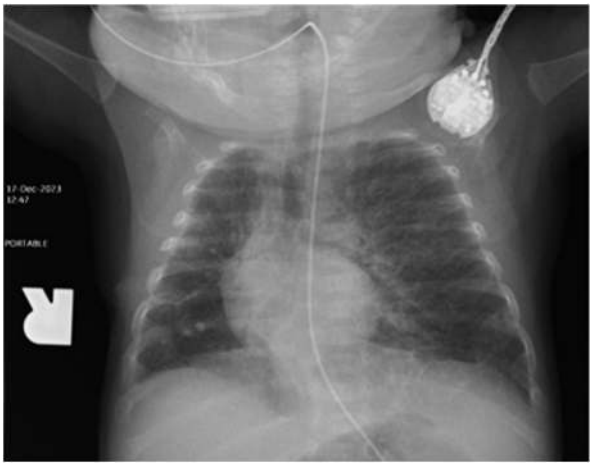


FIGURE 3. Frontal view of the chest on day 53 of life. Patient with status post right lower lobectomy and extubation. There is complete resolution of multicystic hyperinflation of the right lung. Mild asymmetric postsurgical right lung volume loss is seen. The left lung is well expanded. Mild bilateral interstitial thickening of chronic lung diseases is present. Overall, lung aeration has improved.

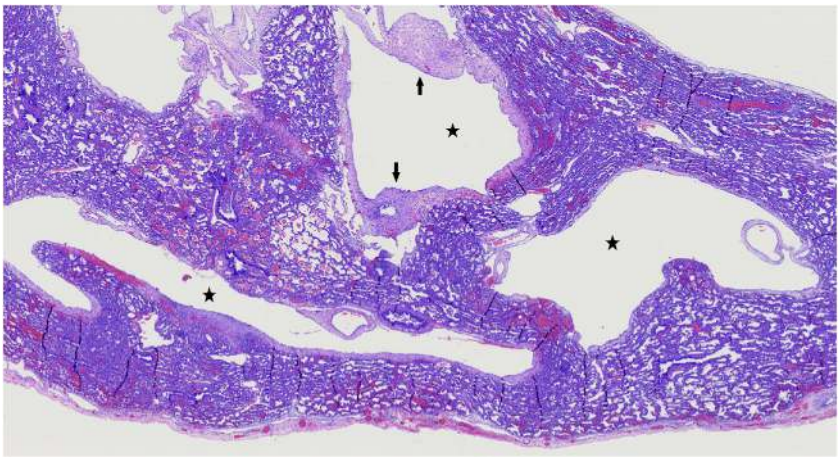


FIGURE 4. The histopathology slide shows airspace enlargement (asterisks) compared with the surrounding lung parenchyma alongside mild fibrotic changes (arrows) consistent with PIE.

- In refractory cases of PIE, surgical consultation may be warranted for potential lobectomy.

American Board of Pediatrics Neonatal-Perinatal Content Specification

- Recognize the clinical, laboratory, and imaging features of air leaks.
- Know how to prevent and manage air leaks.
- Know the appropriate management for an infant with congenital malformations of the lung, including congenital pulmonary lymphangiectasia, and cystic lung diseases, such as CLE, cystic adenomatoid malformation, and mediastinal tumors.

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INDEX OF SUSPICION

Term Infant With Apnea and Seizure-Like Activity

Ina A. Lee, MS,[1] Erin Von Klein, MD,[2] Laura Petrauskas, MD,[3] Gabriella L. Crane, MD,[4] Kevin Louie, DO,[2] Lyndy Wilcox, MD, MMHC[3]

PRESENTATION

The patient is a former 39-week gestational age girl born via spontaneous vaginal delivery to a vaginal delivery to a mother, 35 years of age, gravida 7 para 5015. Pregnancy and delivery are complicated by maternal herpes simplex virus (HSV)-2 (recurrent, with no lesions present at birth), concern for nuchal cord, meconium at birth, and fetal decelerations. Antenatal imaging showed polyhydramnios but was otherwise normal. Apgar scores are 3 at 1 minute, 6 at 5 minutes, and 7 at 10 minutes. The infant is born at a level 1 nursery and requires continuous positive airway pressure (CPAP) and supplemental oxygen after delivery. Due to hypoxia with the patient on CPAP of 5 cm H_2O and fraction of inspired oxygen 0.30, transfer is requested to a higher level of care neonatal intensive care unit (NICU). There is no concern for perinatal depression or hypoxic-ischemic encephalopathy (HIE) reported.

During transport, the infant has 2 desaturation events that resolve with stimulation and increased supplemental oxygen. Upon presentation to the NICU, her initial examination is within normal limits. She subsequently develops recurrent apnea episodes associated with desaturation to 40%; during each episode, the patient is noted to have tensed extremities and rhythmic flexion and extension movements. The NICU team is initially most concerned for seizures and consults neurology for evaluation and placement of video electroencephalography (EEG). An EEG is ordered, and antimicrobials are broadened to include acyclovir for HSV coverage.

The apnea episodes increase in frequency. Therefore, the team proceeds with intubation to protect the patient's airway. Intubation is difficult, requiring more than 3 attempts, with successful intubation by the supervising physician.

ABBREVIATIONS

CPAP	continuous positive airway pressure
NICU	neonatal intensive care unit
HIE	hypoxic-ischemic encephalopathy
EEG	electroencephalography
ENT	otolaryngology
HSV	herpes simplex virus
MRI	magnetic resonance imaging
CT	computed tomography

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DISCUSSION

Differential Diagnosis

The differential diagnosis for apnea and seizure-like-activity in the neonatal population is broad. Most common causes include infections, such as meningitis or sepsis, and neurologic pathologies, such as HIE, mass-effect, bleeding, or stroke. Additional etiologies can include genetic abnormalities such as in-born errors of metabolism, electrolyte derangements, or upper airway obstruction, especially in the setting of syndromes like trisomy 21, Beckwith-Wiedemann, or Treacher Collins.

Diagnosis

The supervising physician notes a round, pink-colored mass along the posterior oropharynx during intubation, leading to consultation of the pediatric otolaryngology (ENT) service for bedside laryngoscopy. The team performs a flexible laryngoscopy, which visualized a mass posterior to the uvula obstructing the choanae.

Computed tomography (CT) demonstrated a large heterogenous naso-oral-pharyngeal mass containing a partially formed tooth, macroscopic fat, and a small amount of enhancing tissue. The mass measures 1.1 × 2.0 × 3.5 cm and shows remodeling of the smooth bone of the posterior nasal cavity. In the meantime, the EEG returned as normal. CT imaging was suggestive of an epignathus, an oropharyngeal teratoma (Figure 1), particularly due to the presence of a tooth. magnetic resonance imaging (MRI) was also obtained to better understand tissue attachments and borders prior to surgery (Figure 2). The mass is removed by ENT (Figures 3 and 4). Pathology demonstrates a mixture of mature fatty tissue, salivary glands, squamous epithelium with keratin pearls, and a focus of dental papillae with enamel epithelium and ameloblasts consistent with a mature teratoma.

The Condition

Oropharyngeal teratomas, also known as epignathus, are a rare form of congenital teratoma originating from the skull base often with attachments at the hard palate or mandible.[1,2] It is a germinal neoplasm consisting of tissues from all 3 germ layers. The incidence of epignathus is estimated to affect between 1 in 35 000 to 1 in 200 000 live births.[3] Depending on their location, these masses may present with dysphagia, rhinorrhea, or nasal blockage; if large, they may cause respiratory obstruction and cyanosis.[4]

Orofacial teratomas identified before birth are often large and can be linked to polyhydramnios, hydrops fetalis, and preterm delivery, leading to a mortality rate of more than 50%.[5] The most serious postnatal complication is severe respiratory distress caused by airway obstruction. Prenatal diagnosis can enable early recognition and anticipatory treatment such as ex utero intrapartum treatment procedures in the most severe cases. However, smaller masses may go undetected in the prenatal period and may present unexpectedly after delivery.

Treatment and Management

It is preferred to detect masses of the airway prenatally to prepare for difficulties with airway at birth. In neonates with obstructive apneas, it is important to prioritize the

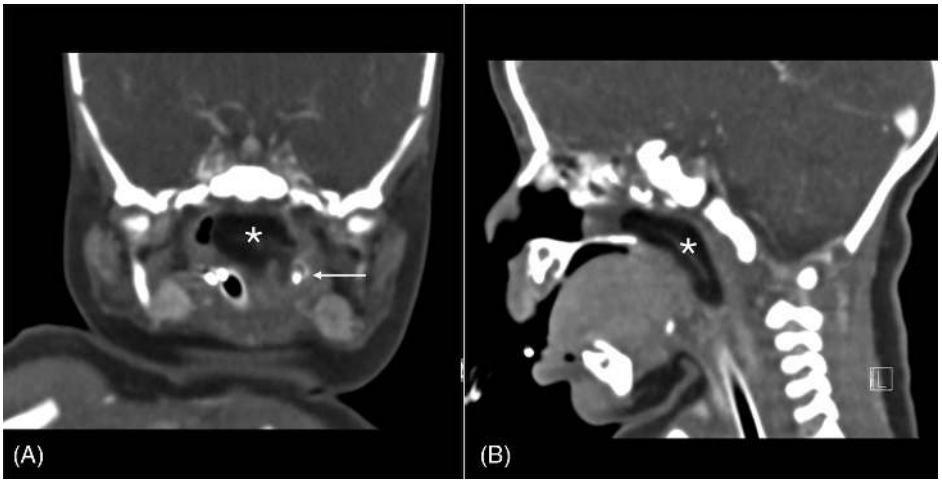


FIGURE 1. Coronal computed tomography (CT) (A) demonstrates a mass (*) occupying portions of the pharynx, which contains markedly hypodense tissue, similar to subcutaneous fat, but with a left lateral soft tissue component containing a tooth (arrow). Endotracheal tube passes beneath the mass. Sagittal CT (B) reveals that the mass (*) causes significant narrowing of the posterior nasopharynx and oropharynx.

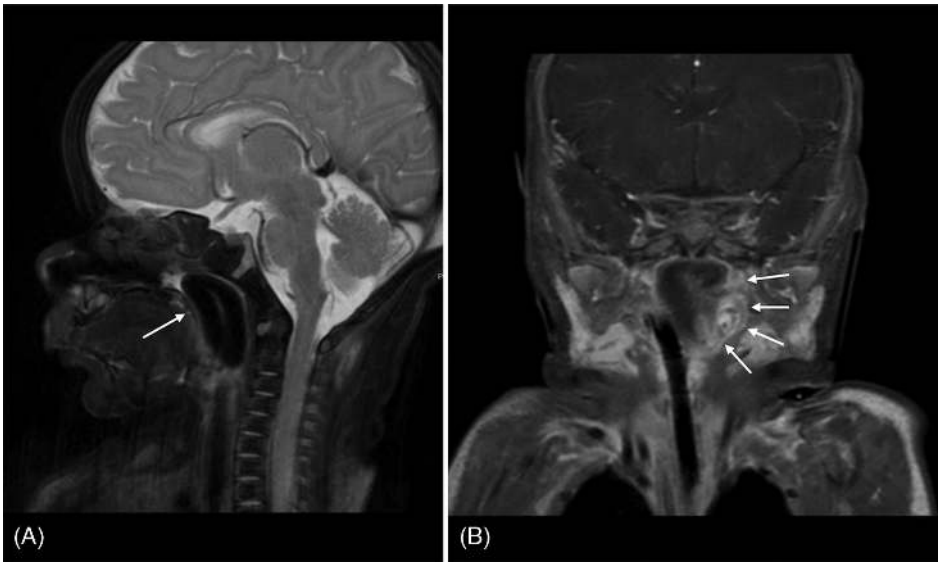


FIGURE 2. On magnetic resonance imaging, sagittal T2-weighted sequence with fat saturation (A) allows clear delineation of the mass as separate from the soft palate (arrow) and highlights the fatty nature of the mass. Coronal T1-weighted post-contrast sequence (B) better defines the enhancing soft tissue component, appearing to arise from the left lateral wall of the pharynx (multiple arrows).

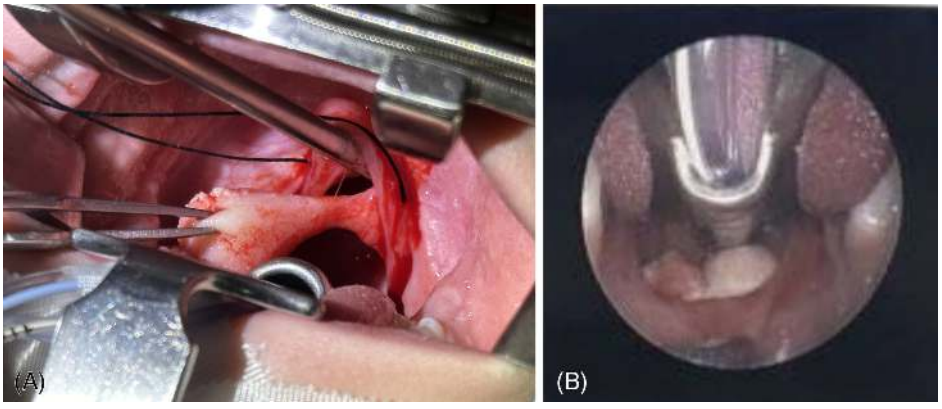


FIGURE 3. (A and B) Visualization of mass in the posterior oropharynx with a rigid 0-degree endoscope performed intraoperatively during mass excision by the pediatric otolaryngology team.

ABCs—airway, breathing, and circulation. Every effort should be made to stabilize the airway. In this case, the mass was noted on intubation. Performing a thorough and full physical examination on all patients is critical. Once the diagnosis of epignathus is determined, management requires multidisciplinary care including NICU and ENT. Further evaluations like flexible laryngoscopy and imaging such as CT and MRI must further delineate the lesion prior to surgical planning. Surgical excision should not be delayed because while the lesions are benign, they may grow quickly and cause further airway destruction and feeding difficulties. There is low concern for recurrence. Scheduling short-term follow-up is appropriate to ensure that the patient has healed from surgery and resumed normal breathing and feeding.

Patient Course

Three days after admission, the patient is taken to the operating room for removal of the mass. General anesthesia was administered. The mass was visualized, confirmed to be attached to the left posterior pharyngeal wall, and removed. The patient is extubated to room air the following day. She had no further episodes of apnea or abnormal movements; the movements were thought to be secondary to discomfort associated with airway obstruction. Oncology is consulted and out-patient follow-up every 3 months for surveillance



FIGURE 4. Gross pathology of mass after surgical excision by pediatric otolaryngology team.

is planned. Upon discharge, the patient has normal work of breathing on room air without noted apneic events since excision.

LESSONS FOR CLINICIAN

- Neonatal epignathus is a true congenital teratoma and may present with prenatal polyhydramnios and obstructive apneas in the neonatal period.
- Consider obstructive pathologies in the differential diagnosis for apnea even in non-syndromic infants.
- Prioritize airway stabilization in neonates with apneas. Potential diagnostic material may be visually noted on intubation.

American Board of Pediatrics Neonatal–Perinatal Content Specification

- Know the various causes of stridor in the newborn and how to assess severity
- Know the clinical manifestations and approaches to therapy of neck masses in the newborn infant

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MATERNAL-FETAL
CASE STUDIES

Managing Fetal Anemia: A Case of Parvovirus Infection in Pregnancy

Rodolfo Fernandez Criado, MD, Stephanie Ros Saposnik, MD

CASE

A gravida 2, para 1-0-0-1 woman aged 27 years with chronic hypertension and a previous cesarean delivery in Brazil presented to her scheduled anatomy ultrasonogram for routine prenatal care at 20 weeks and 1 day's gestation. On imaging, the fetus had ascites and a small pericardial effusion diagnostic of fetal hydrops (Fig. 1). Middle cerebral artery (MCA) Dopplers were performed and noted to be significantly elevated, indicating fetal anemia (Fig. 2). This finding was reviewed with the patient. Her medical and surgical histories were unremarkable, except that she reported having experienced a flu-like illness about 3 to 4 weeks before her ultrasonogram, with symptoms including coughing, sneezing, headaches, arthralgias in bilateral knees, shortness of breath, and lower abdominal pain. The differential diagnosis included an infectious, cardiac, or genetic etiology. The combination of her recent symptomatology and ultrasonogram findings led to a leading differential diagnosis of parvovirus infection. Serum testing with parvovirus serologies was obtained. The parvovirus IgM returned positive, confirming parvovirus infection.

At 20 weeks' gestation, she was transferred to a tertiary care center where she underwent a percutaneous umbilical blood sampling (PUBS) to confirm suspicion of fetal anemia. The PUBS was performed without complication, and fetal anemia was confirmed with a hematocrit of 9.7% (reference range, 35%–48%). The fetus underwent an intrauterine blood transfusion with 15 cc of irradiated, O-negative, cytomegalovirus-negative blood. Several hours after the procedure, she reported diffuse abdominal pain and had decreased fetal movement. Bedside ultrasonogram found absent cardiac activity, confirming intrauterine fetal demise.

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AUTHOR DISCLOSURE: Dr Ros Saposnik is on the board of directors for the Society for Maternal Fetal Medicine. Dr Fernandez Criado has disclosed no financial relationships relevant to this article. This article does not contain a discussion of an unapproved/investigative use of a commercial product/device.

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FIGURE 1. Fetal abdomen with ascites (arrow) as a component of fetal hydrops.



FIGURE 2. Middle cerebral artery Dopplers with elevated value concerning for fetal anemia in the setting of parvovirus infection. The fetal anemia is subsequently confirmed via a percutaneous umbilical blood sampling.

MATERNAL OUTCOME

The patient was counseled extensively regarding mode of delivery, including induction of labor vs dilation and evacuation. She underwent dilation and evacuation procedure under ultrasonogram guidance. She recovered with no physical concerns. She was discharged the same day with physician and social services follow-up.

FETAL AUTOPSY

A fetal autopsy was offered to the patient at the time of counseling for the dilation and evacuation. The patient declined an autopsy after the procedure.

HUMAN PARVOVIRUS B19

Parvoviridae are a class of small, nonenveloped DNA viruses.[1] Human parvovirus B19 is the predominant strain affecting humans. About 30% to 60% of adults have positive antibodies to parvovirus.[2] Similarly, about 35% to 53% of pregnant patients have evidence of immunity from previous infection, given serologic evidence of IgG to the virus.[3] The incidence of B19 infection is highest in schoolteachers (16%) and daycare workers (9%).[4] Notably, the Centers for Disease Control and Prevention (CDC) issued a statement in August 2024 stating there is an increase in the cases of B19 infection in the United States as well as 14 other European countries.[5] Thus, this should increase clinical suspicion for patients with common parvovirus symptoms.

Parvovirus infection can present as erythema infectiosum, or fifth disease, classically characterized with a "slapped cheek" rash on the face and lacy rash along the trunk. Although adults can manifest with a rash, the classic appearance on the face or the trunk is rare. These dermatologic findings are usually accompanied by systemic symptoms, most commonly arthralgias in the adult population. The recommended treatment is routine supportive care, given the virus is self-limiting, especially in immunocompetent individuals.[6] However, in pregnancy, there are unique considerations for the fetus exposed to parvovirus B19.

APPROACHING THE B19-POSITIVE PREGNANT PATIENT

If there is a clinical suspicion of parvovirus B19 in a pregnant patient, assessing serologic IgG and IgM antibodies is recommended.[6] Patients with seropositive IgM antibodies indicate active parvovirus infection. Counseling should be tailored depending on the gestational age at the time of parvovirus diagnosis.[6]

Pregnant patients presenting with active infection before 20 weeks' gestation should be counseled that the PUBS procedure is not typically feasible given limited visualization and the smaller size of fetal structures. Typically, after 20 weeks' gestation, patients may have weekly ultrasonograms to monitor for ultrasonographic evidence of hydrops fetalis (ie, edema, ascites, cardiomegaly, and polyhydramnios). Additionally, MCA peak systolic velocity Doppler is measured to assess anemia, with the anemia characterized as a peak systolic blood velocity more than 1.5 multiples of the median. The usual duration of time for testing is about 10 to 11 weeks after the infection. It is less likely for the fetus to develop fetal hydrops if the infection has not occurred 8 weeks after infection in the pregnant person.[6] Susceptible pregnant patients (no history of exposure, schoolteachers, and daycare workers) should be recommended to engage in proper hand hygiene and to avoid sharing food or drinks.[6]

HYDROPS FETALIS

Hydrops fetalis is a known but fortunately infrequent result of parvovirus B19 infection in pregnancy. Hydrops fetalis is ultrasonographic evidence of fluid accumulation around the soft tissues and serous cavities of the fetus.[7] There is a wide variety of possible causes. These include immune-mediated (eg, isoimmunization) and nonimmune-mediated causes, such as congenital infections, abnormalities in hemoglobin function, or anatomic malformations such as congenital heart or lung defects. Parvovirus B19 infection is cytotoxic to red blood cells, which results in profound fetal anemia. The likelihood of fetal loss is directly correlated with the severity of the anemia in the fetus.

Fortunately, most cases of parvovirus infection in pregnancy resolve spontaneously without fetal infection or fetal sequelae. Studies suggest the rate of hydrops fetalis associated with parvovirus B19 infection before and after 20 weeks of gestation is 11% and less than 1%, respectively.[8] One study from Sweden demonstrated that the risk of third trimester stillbirth from B19 infection was very low, citing 93 deaths out of 33 759 deliveries.[9] Additionally, infection in the first trimester will increase the risk of adverse outcomes.

MANAGEMENT OF ANEMIA AND HYDROPS FETALIS

A fetus with evidence of mild-to-moderate anemia is usually self-limited and typically resolves spontaneously without long-term sequalae. Because the anemia caused by parvovirus is transient, there is low utility in determining the fetal hemoglobin levels, unless there is ultrasonographic evidence of hydrops. Two of the primary modalities for monitoring fetal anemia include the MCA peak systolic blood velocity and ductus venosus velocity; the MCA is usually preferred given its accessibility for measurement via ultrasonogram.

Patients with elevated MCA Doppler ultrasonograms are recommended to have a PUBS procedure, in addition to an intrauterine fetal blood transfusion. The evidence demonstrates that intrauterine transfusion improved the survival rate of fetal parvovirus infection to 82% with transfusion vs 55% without intrauterine transfusion.[10] Although intravenous immunoglobulin has been used to treat patients infected with parvovirus B19, there is no recommendation for its use in the management of infected pregnant patients outside of a research protocol.

Given the high risk of neonatal compromise in the setting of hydrops, it is critical to have coordinated efforts between the obstetrics team and the neonatologists arrange for a coordinated delivery planning with prepared respiratory support and mechanical ventilation. Additional preparation for resuscitation includes paracentesis, thoracentesis, or infusion of blood products, depending on the gestational age and severity of clinical manifestations of the parvovirus infection in the infant. Additionally, mental health support should be considered for the parents. Fetal hydrops can be psychologically altering events, and mental health should be prioritized postpartum.

SUMMARY

A fetus can succumb to complications stemming from parvovirus-induced hemolytic anemia at a previable gestational age. Although most parvovirus infections do not cause anemia and hydrops, a minority of parvovirus infections in pregnancy will result in adverse outcome for the fetus despite appropriate surveillance and treatment.

American Board of Pediatrics Neonatal-Perinatal Content Specification

- Development of immune defenses in the fetus and neonate.
- Assessment of fetal well-being.

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VISUAL DIAGNOSIS

A Late Preterm Neonate With a Small Chest

Abhilasha Gupta, MD, Prashanth Ranya Raghavendra, DM, Sruthi Nair, DrNB, Anitha Haribalakrishna, MD, FRACP

CASE

A late preterm neonate with a small chest.

PRENATAL AND BIRTH HISTORIES

- Born to a 30-year-old gravida 2, para 1 woman.
- A 20-week scan was normal except for the possibility of bilateral short femurs; additional fetal ultrasonogram scans showed similar findings with no fetal growth restriction.
- The parents were nonconsanguineous.
- A previous sibling is aged 5 years and healthy.
- Prenatal maternal laboratory tests returned normal results. No other abnormalities were detected by ultrasonography. The umbilical cord Doppler scan result was normal, and there was no polyhydramnios.
- The estimated gestational age was 36 weeks' gestation.
- This was a spontaneous preterm labor with no additional risk factors for sepsis.
- Born in a lower-segment Cesarean delivery because of previous lower-segment Cesarean secondary to cephalopelvic disproportion.
- The neonate cried immediately after birth with good tone.
- The Apgar scores were 9 and 9 at 1 and 5 minutes, respectively.
- Respiratory distress was noted soon after birth.

PROGRESSION (SOON AFTER BIRTH)

Vital Signs

- Heart rate: 150 beats per minute

ABBREVIATION

SD skeletal dysplasia

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- Respiratory rate: 72 breaths per minute
- Blood pressure: 62/42 (mean 50) mm Hg
- Oxygen saturation: 88% (in room air)
- Temperature: 98.6°F (36.5°C)

Physical Examination

- Birthweight: 2100 g (10th centile as per the modified Fenton's chart)
- Length: 46 cm (30th centile as per the modified Fenton's chart)
- Head circumference: 32 cm (30th centile as per the modified Fenton's chart)
- Head: normocephalic with normal, open, and flat fontanelles
- Face: depressed nasal bridge with no coarse facial features
- Oral cavity: pink mucosae, intact palate, normal sucking, and rooting reflex
- Thorax: bell-shaped, small thorax with pectus carinatum (Figure 1A)
- Lungs: moderate respiratory distress with intercostal and subcostal retractions; bilateral air entry normal
- Cardiovascular: normal S1 and S2, regular rate and rhythm, and no murmurs or gallops
- Abdomen: protuberant, soft, and nontender; normal bowel sounds; and no hepatosplenomegaly (Figure 1A)
- Genitourinary: normal male genitalia and patent anus
- Skeletal: spine appears normal
- Limb examination: no shortening of limbs. No excess skin folds, no evidence of fracture, no congenital talipes equinovarus, and no contractures
- Hip examination: no developmental dysplasia of the hip and no limb-length discrepancy
- Skin: no icterus, birthmarks, or other rashes

PROGRESSION

The neonate was admitted to the neonatal intensive care unit. At age 5 minutes, he was placed on continuous positive airway pressure with fraction of inspired oxygen of 0.25 and peak end expiratory pressure of 5 cm H_2O. A venous blood gas test was normal with a pH of 7.35, partial pressure of carbon dioxide of 39 mm Hg, partial pressure of oxygen of 60 mm Hg, and bicarbonate level of 23.4 mEq/L. A chest radiograph was obtained (Figure 2). He was started on gavage feedings. Detailed anthropometry and body measurements showed that he had a small chest with normal limb length with findings summarized in Table 1. A skeletal survey was obtained (Figures 3A and B) and genetics was consulted.

DIFFERENTIAL DIAGNOSIS

- Asphyxiating thoracic dysplasia (dystrophy)
- Thoracolaryngopelvic dysplasia (Barnes syndrome)
- Schneckenbecken dysplasia
- Fibrochondroplasia
- Jansen metaphyseal dysplasia

The skeletal survey (Table 2) demonstrates the following findings: elevated clavicles, hypomineralized bones with cupping of the distal ends of the radii, a bell-shaped thorax, a small and narrow pelvis, bowing of the femurs, and thoracic scoliosis. Because of the potential of having a skeletal



FIGURE 1. Clinical features of thoracicpelvic dysostosis in the index neonate. (A) Bell-shaped thorax and protuberant abdomen (black arrows). (B) Neonate at the time of discharge at age 16 days with small thorax (black arrow).



FIGURE 2. Radiograph at the time of admission to the neonatal intensive care unit showing mild surfactant deficiency and no pulmonary hypoplasia.

dysplasia (SD) associated with airway abnormalities, a laryngoscopy is done that shows no evidence of laryngeal stenosis. The infant's liver function tests, blood urea nitrogen and creatinine levels, ultrasonogram scans of the head and abdomen, and echocardiography were obtained to rule out systemic associations; these were all found to be normal. Serum calcium, phosphorous, and alkaline phosphorus levels were normal. In consultation with radiology and genetics, a clinical diagnosis of thoracopelvic dysostosis was made.

A detailed evaluation of the parents was done, which showed a small pelvis in the mother with a normal thorax. The radiograph of the mother's pelvis (Figure 3C) showed that the transverse diameter of the inlet, the mediolateral diameter of the midplane, and mediolateral diameter of the outlet were less than 1 unit (ie, 10 cm, normal 15 cm), suggestive of a small pelvis and, given the mother's height of 141 cm, contributing to cephalopelvic disproportion. The skeletal surveys in the father and sibling were normal.

TABLE 1. Detailed Anthropometry and Body Measurements

Measurement	Infant's Value	Interpretation of Infant's Measurement
Chest circumference, cm	25	Less than −2 SDs
Upper arm length, cm	Right: 9 Left: 9	Mean to +2 SDs
Forearm length, cm	Right: 7.5 Left: 7.5	Mean to +2 SDs
Hand length, cm	Right: 5 Left: 5	Mean to +2 SDs
Upper thigh length, cm	Right: 10 Left: 10	Mean to −2 SDs
Leg length, cm	Right: 9.5 Left: 9.5	Mean to −2 SDs
Foot length, cm	Right: 7 Left: 7	Mean to −2 SDs
Upper and lower segment ratio	1.72:1	Normal

Abbreviation: SD, standard deviation.

PRESUMED DIAGNOSIS

Thoracopelvic dysostosis (variant of Barnes Syndrome)

CASE PROGRESSION

The infant's respiratory distress was thought to be most likely caused by mild surfactant deficiency. He is weaned off respiratory support at age 3 days and then started to feed orally. The genetics team met with the family and explained the need for genetic confirmation; the family deferred genetic testing initially because of financial constraints. He was discharged home at age 16 days (Figure 1B) at 38 weeks 2 days postmenstrual age with a weight of 2180 g exclusively breastfeeding. He has follow-up with his pediatrician and genetics.

WHAT THE EXPERTS SAY

SD refers to a group of disorders with abnormalities in the structure and growth of bone and cartilage with an overall prevalence of 2.3 to 7.6 per 10 000 births.[1] The major contributing factor to mortality in cases of SD is the degree of thoracic underdevelopment and pulmonary hypoplasia. The extensive involvement of the vertebrae and associated rib anomalies result in thoracic case deformity and improper ventilation.

Thoracolaryngopelvic dysplasia, also known as Barnes syndrome, is an SD whereby affected infants have a small thorax with short ribs and a small pelvis with laryngeal stenosis.[2] Barnes syndrome has an autosomal dominant pattern of inheritance with 9 cases (3 female adults and 6 infants) reported to date globally and an estimated prevalence of less than 1/1 000 000.[2]

The hallmark feature of Barnes syndrome is a small, rigid bell-shaped chest with short ribs and a small pelvis with a normal height and limb length. Among the 3 women reported,[3–5] all underwent cesarean section for cephalopelvic disproportion, similar to the mother of our patient. However, in contrast to the reported cases, our patient did not have laryngeal stenosis and did not require prolonged ventilation or tracheostomy, suggesting that this infant probably has a variant of Barnes syndrome (ie, thoracopelvic dysostosis without laryngeal stenosis). Among the previously reported 6 infant cases, all required a tracheostomy in early infancy.[3–5] In addition, 1 died in the neonatal period and the

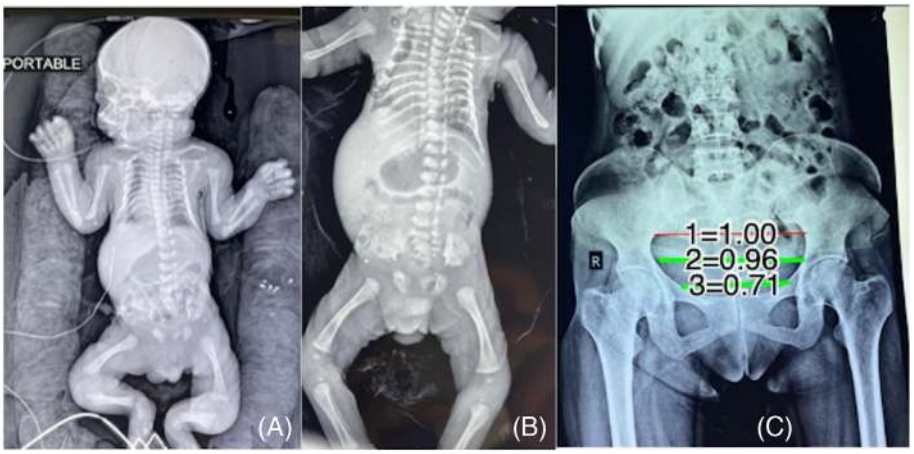


FIGURE 3. (A) Skeletal survey of the index neonate at age 4 days demonstrating elevated clavicles (black arrow), bell-shaped thorax with short horizontal ribs (black asterisk), cupping of the distal end of the left radius (white asterisk), and scoliosis (black arrowhead). (B) Additional radiograph of index neonate at age 4 days showing small and narrow pelvis (black asterisk) and hypomineralized femurs with bowing at the medial ends (black arrow). (C) Radiograph of the index neonate's mother showing a small and narrow pelvis (1, pelvic inlet; 2, midplane; and 3, pelvic outlet).

other in early adolescence after multiple chest expansion surgeries and numerous hospitalizations for recurrent chest infections.[3–5] Long-term data of the remaining 4 infants are not available.

The patient in this case required noninvasive support for a short period and remained hemodynamically stable. Because this infant did not have major involvement of the chest and pelvis, relative sparing of the long bones, absence of respiratory infections after discharge, and normal growth, this infant is likely to have a variant of Barnes syndrome that is probably associated with a better quality of life and prognosis than patients with the originally described syndrome. This patient still requires genetic testing to confirm the diagnosis and increase our understanding of the molecular basis of the variation in presentation.

The other potential diagnoses considered in this case were ruled out clinically and radiographically. Infants with asphyxiating thoracic dysplasia have bowed extremities with a trident pelvis, polydactyly, short limbs, and associated renal and cardiac malformations in addition to short ribs.[6] The absence of rhizomelic shortening of long bones, distinct facial features, and splaying of the end of ribs and spurs arising from the iliac bones makes fibrchondroplasia and Schneckenbecken dysplasia less likely.[7] Jansen metaphyseal dysplasia is also of low probability because affected patients have predominant involvement of the long bones, which was not evident in this patient.[7]

TABLE 2. Skeletal Survey of the Neonate (Figure 3)

Bone Location	Interpretation
Skull	Normal
Clavicle	Elevated location
Ribs and thorax (Figure 2)	Bell-shaped thorax with short horizontal ribs; adequate pulmonary expansion
Humerus, radius, and ulna	Normal appearance with hypomineralized bones; cupping of distal end of left radius
Pelvis	Small and narrow pelvis
Femur and tibia	Normal with hypomineralized bones with bowing of medial end
Phalanges of hand and feet	Normal with hypomineralized bones
Spine	Thoracic scoliosis

TAKE-HOME LESSONS

- A variant of thoracolaryngopelvic dysplasia (Barnes syndrome) should be suspected in neonates with a bell-shaped thorax and maternal-infant dyad having small pelvis and no laryngeal stenosis.
- SDs without pulmonary hypoplasia and other systemic manifestations are less lethal.
- A detailed clinical examination and skeletal survey of a neonate with an SD as well as their parents can aid in the clinical-radiological diagnosis.

American Board of Pediatrics Neonatal-Perinatal Content Specification

- Recognize the diagnostic implications of single vs multiple anomalies.

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OUTCOMES OF NICU GRADUATES

Preterm Infants With Bronchopulmonary Dysplasia and Pulmonary Hypertension

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CASE PRESENTATION

We present the case of a male infant born preterm with a postnatal course complicated by grade 3 bronchopulmonary dysplasia (BPD) and pulmonary hypertension (PH) who was followed in our multidisciplinary neonatal intensive care unit (NICU) follow-up clinic.

The infant was born at $25^{1}/_{7}$ weeks' gestation to a 28-year-old gravida 1 para 0 woman via vaginal delivery secondary to preterm labor and concern for chorioamnionitis. Pregnancy was complicated by subchorionic bleeding noted on 20-week ultrasonogram. The pregnant woman had received an incomplete course of betamethasone prior to delivery. Delivery was significant for meconium-stained amniotic fluid. The infant was initially vigorous with good tone but quickly developed poor respiratory effort and hypoxemia requiring intubation in the delivery room. The infant's Apgar scores were 7 and 8 at 1 and 5 minutes of age, respectively. The infant's birth weight was 760 g, which was appropriate for gestational age. He was admitted to a level III NICU for further management.

Upon admission, he received a dose of surfactant and was extubated to continuous positive airway pressure the following day. In the first week of age, he received a course of ibuprofen to facilitate patent ductus arteriosus (PDA) closure that was diagnosed on echocardiography. He was reintubated and placed on conventional ventilation at 6 days of age following multiple apnea and bradycardia events. Three days later, he was found to have a total parenteral nutrition–related pericardial effusion with cardiac tamponade that resolved after pericardiocentesis and removal of the peripherally inserted central line. At 12 days of age, he had worsening respiratory failure, and he was treated with 14 days of antibiotics for culture-positive *Escherichia coli* bacteremia and pneumonia. At 31 days of age, systemic

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dexamethasone via the Dexamethasone: A Randomized Trial protocol[1] was initiated to facilitate weaning of respiratory support, but it was discontinued early due to worsening respiratory failure requiring escalation to high-frequency oscillatory ventilation. Infectious workup at this time was notable for sputum culture positive for *E. coli* and *Stenotrophomonas*, and after completion of antibiotic therapy, he transitioned to conventional ventilation. An additional course of systemic dexamethasone was initiated at 54 days of age. He weaned on respiratory support throughout his course but ultimately was unable to be extubated. During this time, diuretics were initiated. At $35^{6}/_{7}$ weeks' postmenstrual age (PMA), he had a failed extubation attempt. At 36 weeks' PMA, he was on invasive mechanical ventilation and met Jensen criteria for grade 3 BPD. He remained on the conventional ventilator until arrival at our facility.

He transferred to our facility at 39 weeks' PMA for potential PDA device closure and further management of chronic lung disease. Plain chest radiograph was obtained on admission (Figure 1). The PDA was found to have spontaneously closed. He was initiated on an additional course of systemic dexamethasone on DOL 99 and extubated from conventional ventilation to continuous positive airway pressure. At 44 weeks' PMA, a follow-up echocardiogram was performed due to his continued tachypnea and need for respiratory support. He was found to have a new diagnosis of right ventricular hypertension based on interventricular septum position during systole and tricuspid regurgitation pressure gradient of 41 mm Hg (normal <32 mm Hg) (Figure 2). Over the course of 3 months, he slowly weaned to 0.25 L per minute (LPM) supplemental oxygen via nasal cannula (NC). Given his overall clinical appearance and ability to wean on oxygen support, he was not initiated on pulmonary vasodilator therapy, and echocardiograms remained stable. At time of discharge, he was on 0.25 LPM supplemental oxygen via NC and being treated with budesonide and chlorothiazide.

His overall NICU course was also significant for feeding intolerance requiring continuous postpyloric feedings. He was slowly transitioned to nasogastric (NG) feeds, and by the time of discharge, he was tolerating a combination of



FIGURE 1. Plain chest radiograph on level IV neonatal intensive care unit admission with findings consistent with bronchopulmonary dysplasia. Endotracheal tube tip in mid thoracic trachea. Postpyloric feeding tube in place. Evidence of bilateral chronic lung disease with coarse opacities evident, right greater than left.

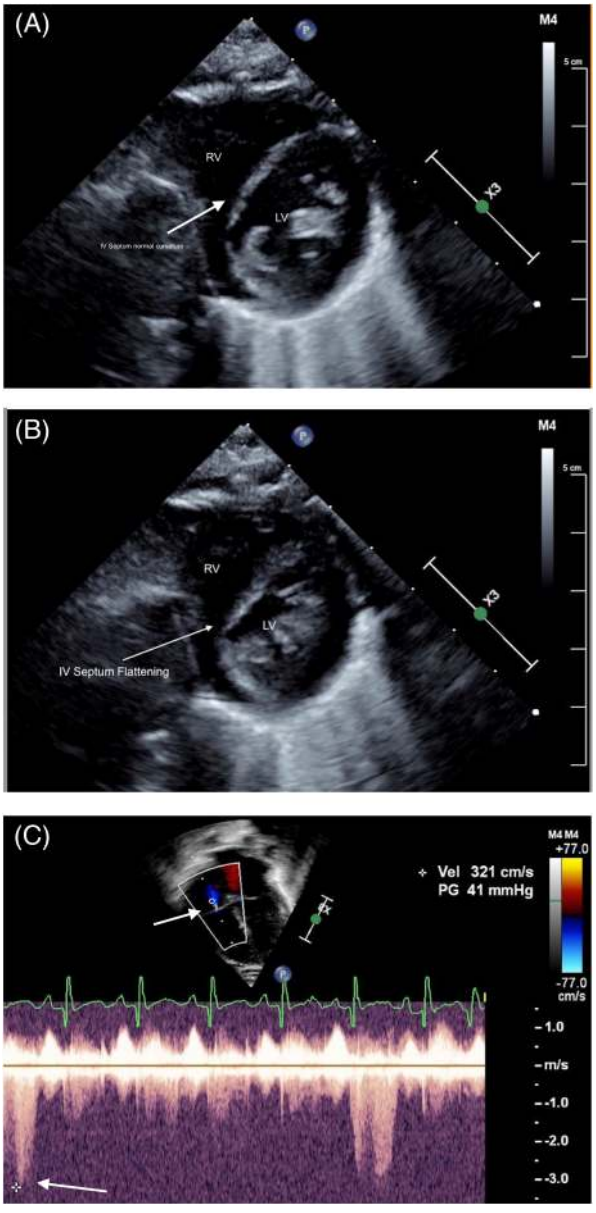


FIGURE 2. (A) Echocardiographic evidence of normal interventricular (IV) septal curvature. (B) Echocardiographic evidence of IV septal flattening in systole. (C) Tricuspid regurgitation demonstrated on echocardiogram with measured pressure gradient (PG) of 41 mm Hg. LV, left ventricle; RV, right ventricle; Vel, velocity.

oral feeding and NG gavage feedings. The family received education on NG insertion prior to discharge. Multiple head ultrasonograms were negative for intraventricular hemorrhage, and his retinas matured appropriately without intervention. He passed his hearing screen bilaterally prior to discharge.

At the time of discharge, the infant was 6 months old in chronologic age, or 51 weeks' PMA. Prior to discharge, the team discussed with the family that he required close outpatient monitoring and management of numerous health concerns. It was anticipated that he would slowly be able to wean off all oxygen support and diuretics. Cardiology would continue to follow his PH closely. His family was counseled that he may require a gastrostomy tube in the outpatient setting if his oral feeding did not progress. Scheduled discharge follow-up included a multidisciplinary BPD clinic, cardiology, occupational therapy for developmental and oral feeding support, and a referral to early intervention.

FOLLOW-UP COURSE

The child is now 5 years old, and he was followed in the BPD multidisciplinary clinic until the age of 3 years. The clinic team included neonatology, pulmonology, occupational therapy, physical therapy, and speech therapy. He also was followed separately by cardiology for PH management.

Due to his severe BPD, the decision to wean his respiratory support as an outpatient was made in collaboration between BPD and cardiology clinic providers. Three weeks after discharge, he was weaned from 0.25 LPM NC to 0.125 LPM NC. Approximately 1 month later, he weaned off supplemental oxygen during the day and remained on 0.125 LPM at night.

Echocardiography 4 months after discharge showed continued mild right ventricular hypertension; therefore, cardiology recommended he not be weaned further and remain on his nocturnal supplemental oxygen. A nocturnal home oximetry study was then performed in room air at 7 months' corrected age, which showed no oxyhemoglobin desaturation below 90% and an oxyhemoglobin desaturation event index of 2.4 (Figure 3). The reference values for this index are not officially established in former preterm infants and are under investigation in infants aged less than 1 year, but evidence shows it would typically be less than 5 to 10 at this age in healthy infants. A repeat echocardiogram at 8 months' corrected age showed resolution of right ventricular hypertension, and he was subsequently weaned off supplemental oxygen support. Chlorothiazide was discontinued at 5 months' corrected age, and budesonide was discontinued at 8 months' corrected age. He did not require albuterol after discharge. An echocardiogram was completed off supplemental oxygen, which continued to show no evidence of right ventricular hypertension. He never required pulmonary vasodilators, and he had no respiratory-related readmissions.

From a feeding perspective, he progressed to full oral feedings and his NG tube was removed within 1 week of discharge. He continued to advance in his oral motor skills appropriately and did not require feeding therapy. He demonstrated adequate growth postdischarge, and at his last NICU follow-up clinic appointment, his weight was in the 85th percentile, and length was in the 88th percentile. Due to NICU stay greater than 5 days, audiology follow-up was obtained.

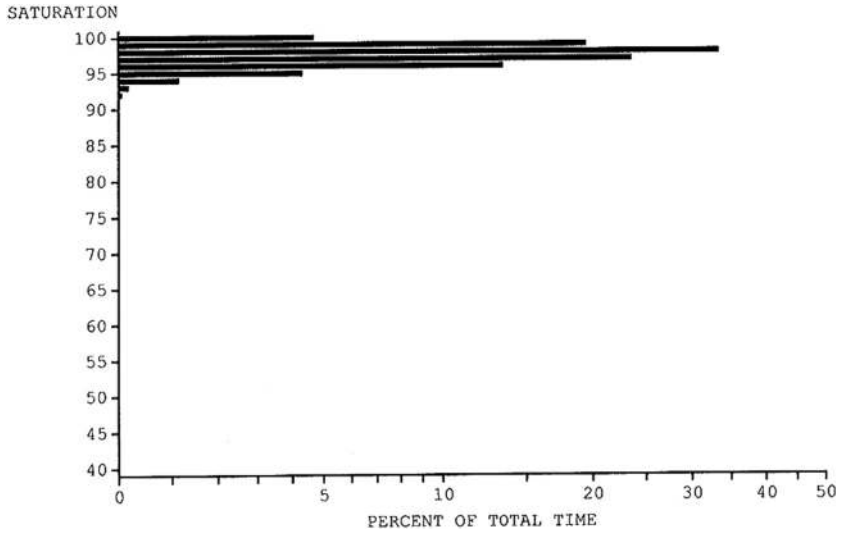


FIGURE 3. Overnight oximetry study in room air. This demonstrates that the patient is spending <2% of the night saturating <95%, with no evidence of desaturations <92%.

On a routine otoacoustic emissions test at 14 months' corrected age, there was concern for abnormal cochlear function of his right ear, but an auditory brainstem response evaluation was performed and was normal bilaterally.

At 43 weeks' PMA, he had poor repertoire writhing movements on a general movements assessment, but at 51 weeks' PMA, he had an assessment that demonstrated normal age-specific fidgety movements. He was followed by outpatient occupational therapy twice a month from the time of discharge until approximately 9 months' corrected age and by early intervention physical therapy from 5 to 12 months' corrected age. He was discharged from both disciplines due to appropriate developmental progression. Per family report, there were no concerns about developmental delay at follow-up visits. At 22 months' corrected age, concern was raised for expressive language delay at a routine developmental follow-up appointment with neonatology. He underwent Bayley-III Developmental Assessment at 25 months' corrected age. This revealed he was delayed in all 3 categories: cognitive (composite score 60, 0.4 percentile), language (composite score 68, 2nd percentile), and motor (composite score 79, 8th percentile). He was referred to outpatient speech therapy and received therapy biweekly until he started preschool, at which time his services were transitioned to school-based therapy. He is currently in kindergarten and continues to have evidence of global developmental delay. He is receiving speech therapy and occupational therapy and has an individualized education plan. He enjoys school and is continuing to make developmental progress.

Overall, he has been in great health since discharge from the NICU. He had 1 emergency department visit at 12 months' corrected age for right acute otitis media and left lower lobe pneumonia, which resolved with amoxicillin. He had snoring, chronic nasal congestion, and recurrent right otitis media and so ultimately underwent bilateral adenoidectomy and right myringotomy at 15 months' corrected age with resolution of symptoms. He continues to follow up with pulmonology annually and currently does not require any medications. He had 1 final echocardiogram at 2 years of age that continued to show no evidence of PH; therefore, he was discharged from the cardiology clinic.

EXPERT DISCUSSION

Respiratory Outcomes

BPD is the most common pulmonary morbidity related to prematurity.[2] BPD arises from abnormal postnatal development of the lung and is defined by alveolar simplification, fibrosis, inflammation, and abnormal vascular development. Infants with BPD are at increased risk of poorer pulmonary outcomes compared with premature infants without a diagnosis of BPD, including increased emergency department visits, hospital readmissions, health care utilization, and requirement of respiratory support after initial hospital discharge.[3,4] Rates of discharge to home on supplemental oxygen vary from 28% to 50% of premature infants with BPD.[5,6] For patients with BPD who are discharged on supplemental oxygen, reported duration of home oxygen needs is as short as 2 to 3 months to more than 12 months of age.[7–9] Those discharged on supplemental oxygen have even further increases in rate of respiratory rehospitalization, total number of hospitalizations, asthma or BPD medication use, and home nursing care in the first 2 years of age.[10] Infants born <1250 g with severe BPD requiring supplemental oxygen at the time of discharge are more likely to require respiratory medications than both survivors with less severe BPD and survivors without BPD at 3-year follow-up.[11]

Infants with BPD are also at increased risk of sleep-disordered breathing, including obstructive sleep apnea, central sleep apnea, hypoventilation, and nonapneic hypoxemia,[12] and may require polysomnography as an outpatient prior to being weaned off supplemental oxygen. It has been reported that infants with BPD who require home supplemental oxygen have more abnormalities on polysomnography, particularly increased central events, compared with infants with BPD who are discharged in room air.[13] The risk of airway obstruction and/or obstructive sleep apnea due to prematurity may even persist until adulthood.[14] The presence of sleep-disordered breathing may increase the duration of time that an infant is required to remain on supplemental oxygen after discharge. The patient described in this case required an adenoidectomy after discharge due to adenoid hypertrophy with associated snoring, but there have not been published reports documenting increased rates of tonsillectomies or adenoidectomies in former preterm infants, and predischarge magnetic resonance imaging performed in preterm and full-term newborns did not demonstrate differences in adenoidal or tonsillar volumes at near-term and term PMAs.[15]

Beyond infancy, children with BPD have higher rates of wheezing and asthma compared with preterm survivors without BPD and are more likely to use bronchodilators up to the age of 2 years, with more persistent wheezing between 2 and 5 years.[3,4] Rates of rehospitalization, wheezing/asthma, and medication use in former premature infants decrease stepwise in the first 5 years of age and beyond,[3,16] but symptoms may continue and remain higher in children with BPD than those without. Children with BPD are 4.5 times more likely to have wheezing and chronic cough than matched preterm controls without BPD at 9.5 years

of age.[17] Compared with 0 control patients, a quarter of these children are also receiving asthma medications at this age. On the contrary, an additional study found that prematurity alone is associated with increased risk of hospitalization and respiratory complaints between ages 5 and 11 years, but having a diagnosis of BPD was not significantly associated with risk.[16] Not only do these children potentially remain symptomatic, but those with BPD have persisting structural abnormalities in their lungs on computed tomography (CT). Abnormalities on CT were detected in 81% of school-aged children, with most abnormalities in the children who had the most severe BPD, which may imply continued long-term pulmonary symptoms.[18]

Children with BPD have consistently been shown to have lung function impairment throughout childhood and adolescence that is primarily characterized by obstructive respiratory disease, although some children have a mixed obstructive and restrictive pattern.[19–21] Furthermore, former preterm infants with and without BPD have impaired diffusion capacity and reduced exercise tolerance even into adulthood.[22,23] Perhaps more concerning, former preterm infants with BPD have early-onset decline in lung function by the teenage years[24]; the combination of decreased lung function and early-onset decline in lung function sets these vulnerable children up for the development of early-onset chronic obstructive pulmonary disease.[25] Despite the significant ongoing respiratory challenges for these patients throughout their life span, there are no specific therapies for impaired lung function in BPD, although 1 clinical trial showed improvement in expiratory flow rate with a combination of inhaled steroids and long-acting beta agonists.[26]

Several long-term studies have demonstrated that infants with BPD have abnormal pulmonary function into adulthood as well as increased risk of pulmonary vascular diseases.[27] Duration of mechanical ventilation in the neonatal period is the strongest predictor of mean pulmonary artery pressure in adults. Adults who were born premature are also at increased risk of developing hypertension, glucose intolerance, and congestive heart failure.[28,29] Due to these findings, it is imperative that patients with a diagnosis of BPD have lifelong follow-up with a pulmonologist.

Cardiovascular Outcomes

Along with adverse pulmonary outcomes, infants with BPD may have comorbid cardiovascular disease. In a study reported from the Children's Hospital Neonatal Consortium, 22% of patients with severe BPD had concomitant PH.[30] The diagnosis of PH is typically made via transthoracic echocardiography during the neonatal intensive care hospitalization, and screening should occur in any infant with established BPD at 36 weeks' PMA as well as prior to discharge. The etiology of BPD-related PH is likely multifactorial, but it is hypothesized to be related to hypoxia/hyperoxia-induced vascular remodeling, persistent hypercapnia, vascular growth arrest, and pulmonary overcirculation related to intra- or extracardiac shunts that promote pulmonary vasoconstriction.[31] In addition, in the BPD lung, intermittent hypoxemia adversely affects alveolar development and vascular remodeling and growth.[32] Infants with BPD born at younger gestational ages and lower birth weights are at highest risk of PH.[33] Risk of PH increases significantly with the severity of BPD from 6%, 12%, and 39% for those with mild, moderate, and severe BPD, respectively.[34] Infants with BPD-related PH can be managed medically with supplemental oxygen and/or pulmonary vasodilators. The American Thoracic Society clinical practice guideline for home oxygen therapy for affected children strongly recommends home oxygen therapy be prescribed in children with PH. This is due to the potential benefit of lessening the undesirable consequences of chronic hypoxemia because it directly interrupts the vicious cycle of PH-causing hypoxemia, which in turn worsens the PH.[35] PH can also be managed with pulmonary vasodilators. Up to 11% of patients with severe BPD require a pulmonary vasodilator at discharge,[36] and single therapy with sildenafil is the most commonly used vasodilator.[37]

Infants with BPD-associated PH are at even higher risk of suboptimal outcomes. Infants with BPD-related PH are at risk of increased mortality overall, with a reported mortality rate of up to 48% within 2 years of PH diagnosis.[38] A meta-analysis of preterm infants with BPD-related PH reports a long-term follow-up mortality rate of 40%, with an overall almost 5 times increased relative risk of death compared with those with BPD and no PH.[34] Of the infants who die with BPD-related PH, 24% die after discharge from their initial hospitalization.[39] BPD with PH is also associated with additional respiratory and feeding support requirements at discharge. BPD with PH is associated with higher rates of tracheostomy, supplemental oxygen use, and tube feedings upon initial hospital discharge compared with BPD without PH.[30] PH is also associated with increased frequency of readmission in the first year of age[30] as well as higher oxygen flows at discharge and longer supplemental oxygen needs.[7] It is important for all neonatologists to be aware of these risks to improve counseling of families and to ensure adequate follow-up with PH specialists after discharge.

Fortunately, for infants with BPD-related PH who survive beyond 6 months, the PH typically resolves. Reported PH resolution rates are 47%, 79%, and 94% at 1, 2, and 2.5 years' corrected age, respectively.[40] An additional study examining the postdischarge disease trajectory in 44 survivors with

BPD-related PH found that at 3 years' corrected age, 66% had resolution of their PH.[39] Of the one-third that still had PH, half were still on both oxygen and pulmonary vasodilators, and the remaining were either on oxygen or pulmonary vasodilators alone.[39] This population included patients discharged on mechanical ventilation, and, therefore, the outcomes may describe a more severe population. A separate population had a much shorter duration of vasodilators, with 58% of patients weaning off vasodilators by less than a year of age.[37] The patient described in this case followed the typical trajectory with slight prolongation of supplemental oxygen needs related to his PH but resolution of his PH by 2 years of age.

It is important to mention that there has been growing evidence implicating cardiovascular abnormalities in early adulthood in former preterm infants with BPD. Former preterm infants have higher mean pulmonary arterial pressures with almost half meeting criteria for borderline or overt PH in the third decade of life.[27] There are also noted increases in pulmonary vascular resistance and elastance at rest and during exercise in former preterm adults, and they are less able to augment cardiac index for exercise.[27] As younger and smaller infants are surviving to discharge and into early adulthood, monitoring long-term cardiovascular outcomes will be of the utmost importance, including improving awareness of our adult providers of all implications of being born preterm.

Neurodevelopmental Outcomes

Lastly, infants with BPD are at risk for poorer feeding outcomes and neurodevelopmental impairments. They are at increased risk of feeding difficulties and experience a delay in achieving oral feeding goals for age. Infants with BPD have irregular breathing during oral feeding compared with term infants; therefore, there is a higher risk of swallow dsyfunction.[41] Compared with preterm infants without a diagnosis of BPD, infants with BPD have higher rates of apneic swallows after 35 weeks' PMA and have less coordination of swallowing and breathing during feeding, implying they do not follow expected maturational patterns with oral feeding.[42] But, unlike other diagnoses, infants with BPD with dysphagia or aspiration will typically have a functional swallow by 2 to 4 months' corrected age.[43,44] If there are concerns regarding feeding safety and ability to progress, it is imperative for the patient to have follow-up with feeding therapy. The patient described in this case required discharge with an NG tube but progressed quickly and met feeding goals by about 3 months' corrected age.

Increased rates of cerebral palsy (CP) and neurodevelopmental impairments, including lower cognitive and language scores, are seen in infants with BPD, even after adjusting for major risk factors, including intraventricular hemorrhage or cystic periventricular leukomalacia.[45,46] This may be related to disease severity and brain injury associated with frequent hypoxemic episodes or associated with postnatal therapies such as systemic corticosteroids. In infants that are less than 28 weeks' gestational age at birth, the risk of CP is 6-fold higher for quadriplegic CP and 4-fold higher for diplegic CP at 2 years of age in infants who were intubated at 36 weeks' PMA compared with those without BPD.[47] Infants born <1000 g with a diagnosis of BPD are over 2 times more likely to have any diagnosis of CP, blindness, deafness, or cognitive delay at age 18 months.[48] There are also several reports of persistence of cognitive deficits from early childhood to school age in this population. This includes persistently lower scores on the Mental Developmental Index of the Bayley Scales at the age of 3 years[49] and deficits in general intelligence, reading, mathematics, motor performance, memory, and attention at 8 years of age with higher utilization of special education services compared with both term and preterm peers.[50] Not only do children with BPD have lower scores, but the proportion of children who are significantly delayed are increased in the group with BPD.[50] Interestingly, in this same cohort, of the children with BPD, those who had a history of steroid exposure had lower IQ scores, perceptual organization, motor scores, and higher participation in special education. In addition to motor and cognitive delays, language development is reported to be delayed in children with BPD, including increased risk of significant language delay (<2 SDs below the mean) at age 18 to 22 months[46]; lower receptive, expressive, and total communication competence scores at 3 years of age[51]; and lower scores in both expressive and receptive language at 8 years of age[52] compared with peers without BPD as well as term controls. There have also been reports of difficulties in perceptual motor tasks in patients who required a longer duration of supplemental oxygen in 7- and 16-year-old former very-low-birthweight children.[53,54] Although risks are overall increased in BPD, there have been multiple studies that have demonstrated similar neurodevelopmental outcomes between infants with BPD discharged on supplemental oxygen or breathing room air, including cognitive, CP, and neurosensory impairment.[10,55,56]

Along with more severe respiratory outcomes, premature infants with a diagnosis of PH have significantly lower cognitive (85 vs 95, $P = .004$), language (81 vs 89, $P = .040$), and motor (88 vs 94, $P = .010$) scores of the Bayley-III at 18 to 24 months' corrected age when compared with other preterm infants.[57] In total, 45% of infants with PH met criteria for cognitive delay.[57] In addition, weight z scores and head circumference z scores were significantly lower in those with

PH.[57] In a subgroup analysis of just infants with BPD, the cognitive score was significantly lower, and weight *z* score after discharge was poorer in the BPD + PH group compared with BPD without PH.[57] The patient described in this case had significant cognitive, language, and motor delays at 2 years' corrected age, as would have been expected due to his clinical history.

CONCLUSION

In summary, preterm infants with BPD are at increased risk of suboptimal respiratory and neurodevelopmental outcomes, and PH is a risk factor of worsened outcomes in this population. The evidence suggests the importance of counseling families of these risks to support decision-making and to ensure close medical and developmental follow-up ideally with programmatic multidisciplinary follow-up to ultimately optimize the outcome of preterm infants with BPD and PH. These patients often require follow-up with pulmonology, neonatology, feeding teams, cardiology, and developmental specialists along with multiple therapy disciplines and benefit from lifelong follow-up with pulmonology. It is imperative that neonatologists are aware of these outcome risks in order to appropriately support and counsel families throughout their neonatal intensive care hospitalization and beyond.

American Board of Pediatrics Neonatal-Perinatal Content Specification

- Know the management of BPD/chronic lung disease
- Know the prognosis, long-term complications, and permanent sequelae of BPD
- Know the risks of neurodevelopmental impairments in full-term infants with medical risk factors, such as PH, congenital heart disease, and chronic lung disease

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ANSWER KEY FOR FEBRUARY 2025 NEOREVIEWS

Decoding Hearts: Genetic Insights and Clinical Strategies in Congenital Heart Disease:

1. C; 2. C; 3. E; 4. B; 5. E

Effects of Assisted Reproductive Technology on Genetics, Obstetrics, and Neonatal Outcomes:

1. A; 2. C; 3. D; 4. E; 5. B