Title

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ORIGINAL ARTICLE

PRENATAL DIAGNOSIS WILEY

Retrospective identification of prenatal fetal anomalies associated with diagnostic neonatal genomic sequencing results

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Abstract

Objective: To determine which types of fetal anomalies are associated with postnatal diagnoses of genetic diseases by genomic sequencing and to assess how prenatal genomic sequencing could affect clinical management.

Method: This was a secondary analysis of the second Newborn Sequencing in Genomic Medicine and Public Health study that compared fetal imaging results in critically ill infants who had actionable versus negative postnatal genomic sequencing results.

Results: Of 213 infants who received genomic sequencing, 80 had available prenatal ultrasounds. Twenty-one (26%) of these were found to have genetic diseases by genomic sequencing. Fourteen (67%) of the 21 with genetic diseases had suspected anomalies prenatally, compared with 33 (56%) of 59 with negative results. Among fetuses with suspected anomalies, genetic diseases were 4.5 times more common in those with multiple anomalies and 6.7 times more common in those with anomalies of the extremities compared to those with negative results. Had the genetic diseases been diagnosed prenatally, clinical management would have been altered in 13 of 14.

Conclusion: Critically ill infants with diagnostic genomic sequencing were more likely to have multiple anomalies and anomalies of the extremities on fetal imaging. Among almost all infants with suspected fetal anomalies and diagnostic genomic sequencing results, prenatal diagnosis would have likely altered clinical management.

Key points

What's already known about this topic?

- Diagnostic yield for prenatal genetic testing varies by type of anomaly suspected.
- Genomic sequencing can inform management of critically ill neonates.

The abstract for this paper was presented in poster format (abstract #267) at the 41st Society of Maternal-Fetal Medicine Meeting, which took place from January 25 to 30, 2021, on a virtual format.

What does this study add?

- Diagnostic genome sequencing was associated with multiple fetal structural anomalies.
- The genomic sequencing results, if known prenatally, would have altered management in the majority of cases in this study cohort.
- We also describe prenatal imaging findings in several rare genetic disorders.

1 | INTRODUCTION

Currently, routine prenatal genetic testing is limited to karyotype, fluorescence in situ hybridization (FISH), targeted gene panel, chromosomal microarray, and, increasingly in recent years, whole exome sequencing (WES). Genomic sequencing, which includes WES and whole genome sequencing (WGS), can diagnose approximately 6000 single locus genetic diseases. WGS can also identify copy number and structural variants, a subset of which are detected by chromosomal microarray. Two of the largest cohort studies on prenatal WES to date found similar diagnostic yields of approximately 10% in pregnancies complicated by fetal structural anomalies but with normal karyotype and microarray.[1,2] Other studies reported even higher yields.[3–5] Pregnancy complicated by multiple fetal anomalies is associated with the highest yield for diagnostic genetic variants followed by skeletal anomalies and non-immune hydrops fetalis.[1–3,6] WGS and WES have not been adopted into routine practice in obstetric care due to high cost and suspected low diagnostic yield above karyotype and microarray. Furthermore, accurate interpretation of sequencing depends on phenotype characterization, which is less robust prenatally. Currently, limitations in prenatal phenotype contribute to the suspected poor diagnostic yield. As genomic sequencing becomes more sensitive, more accurate, cheaper, and faster, it will be increasingly utilized in clinical practice. Thus, it is prudent to determine the appropriate populations in which to use these technologies.

Rapid genomic sequencing of infants can diagnose genetic disorders in as little as 19.5 h.[7] In acutely ill infants, rapid diagnosis enables prompt initiation of optimal treatments. This is especially important for the several hundred genetic diseases that have rapid progression and for which effective treatments are available. Rady Children's Institute for Genomic Medicine performed the second Newborn Sequencing in Genomic Medicine and Public Health (NSIGHT2) study to understand the effectiveness and outcomes of rapid genomic sequencing in caring for critically ill infants.[8–10]

The goal of the current study was to determine whether specific types of anomalies detectable on fetal imaging were associated with positive neonatal WES or WGS results and assess if clinical management would have changed if genomic sequencing results were known prenatally among infants. We define change in clinical management as any change in prenatal or immediate postnatal care such as additional prenatal imaging, indicated subspeciality consultations, or delivery location close to level III/IV neonatal intensive care unit (NICU).

2 | METHODS

This was a secondary analysis of the NSIGHT2 study[8] comparing the prenatal imaging studies on critically ill infants who had positive WES/WGS results versus those who had negative genomic sequencing results. The NSIGHT2 study was a prospective randomized controlled trial (RCT) comparing the clinical utility of rapid WGS (rWGS) and rapid WES (rWES) in acutely ill infants. Infants of age less than 4 months were eligible for enrollment if the etiology of their illness was not known within 96 h of admission. Infants with known genetic disorders that explained their clinical diagnosis were excluded. The majority of infants in the cohort were from the neonatal, pediatric, and cardiovascular ICUs at Rady Children's Hospital. Study participants were enrolled between June 29, 2017, and October 9, 2018, and were randomized to rWGS or rWES, except in cases where ultra-rapid WGS (urWES) was performed for extremely ill infants. At least one parent or guardian gave informed consent for participation in the NSIGHT2 study. The protocol for this study was approved by the University of California, San Diego (UCSD) and Rady Children's Hospital Institutional Review Board (IRB #190063X) on February 28, 2019 (ClinicalTrials.gov NCT03211039). Informed consent was waived for the current study given its retrospective nature. All infants from singleton and multiple gestation pregnancies who had received genomic sequencing as part of the NSIGHT2 study were eligible for inclusion. Infants without available prenatal imaging studies in the Rady or UCSD electronic health record were excluded. Maternal demographics collected include age, parity, body mass index (BMI), ethnicity and race, alcohol usage, smoking history, and illicit drug use. We reviewed all available prenatal records, including prenatal genetic testing, obstetrical ultrasounds, and delivery records with particular attention to any available first trimester nuchal translucency ultrasound imaging, midtrimester anatomy survey, fetal echocardiogram, growth ultrasounds, and fetal magnetic resonance imaging (MRI). We recorded any suspected fetal structural anomalies, amniotic fluid index or maximum vertical pocket, biometric parameter measurements of biparietal diameter, head circumference, abdominal circumference, femur length, and estimated fetal weight (EFW). All suspected fetal anomalies were recorded. The only exception were isolated "soft markers" for aneuploidy that are considered a normal variant if isolated and would not require follow-up, that is, isolated echogenic intracardiac focus or choroid plexus cysts.[11] Markers for aneuploidy that may progress or associated with other findings such as thickened nuchal fold, echogenic bowel, or renal tract dilation were recorded. Fetal growth restriction was defined as EFW less than 10th percentile.

Oligohydramnios was defined as amniotic fluid index (AFI) <5 cm or maximum vertical pocket (MVP) <2 cm. Polyhydramnios was defined as AFI > 25 cm or MVP > 8 cm. Antenatal and infant outcomes including gestational age at delivery, mode of delivery, reason for admission to the intensive care unit, and discharge diagnosis were collected. We also collected genomic sequencing results, type of sequencing performed, and the Human Phenotype Ontology (HPO) terms captured neonatally. For the NSIGHT2 study, variants of unknown significance (VUS) were not reported due to the Food and Drug Administration (FDA) requirements for the study. For infants with positive findings on genomic sequencing, we evaluated whether knowledge of genomic sequencing results at time of prenatal imaging studies would have impacted clinical management.

We used Fisher's exact test to compare findings between the two groups and calculated the odds ratio using SPSS version 26 statistical software (IBM Corporation).[12]

3 | RESULTS

3.1 | Study population

Two hundred thirteen infants were enrolled and sequenced in the NSIGHT2 study, of whom 80 had available prenatal ultrasounds to review (Figure 1). Of these, 21 (26%) were diagnosed with genetic diseases by genomic sequencing, with seven through rWES and 14 through rWGS. Fifty-nine infants had negative sequencing results (32 rWES and 27 rWGS). There were no differences identified in maternal and pregnancy demographics between the two groups using Fisher's exact test (Table 1).

Fourteen out of 21 (67%) of infants with positive rWES/rWGS results had suspected anomalies on fetal ultrasound while 33 out of 59 (56%) of infants with negative sequencing had suspected fetal anomalies. Table 2 shows the anomalies by body system seen on prenatal ultrasound between the two groups, and Table 3 lists the type of anomalies by body system. Fetal MRI studies were performed in three singleton pregnancies. All three were done in pregnancies with negative neonatal sequencing. The findings on the three MRIs, lumbar myelomeningocele, agenesis of the corpus callosum, and posterior encephalocele, were concordant with the ultrasound findings.

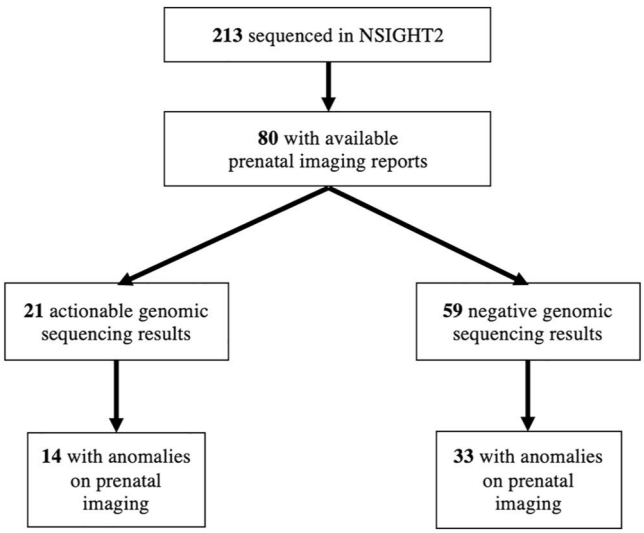

FIGURE 1 Flow diagram of the number of infants in the NSIGHT2 who had available prenatal imaging by genomic sequencing result and anomalies on prenatal imaging

3.2 | Genomic sequencing yield

Among those with suspected fetal anomalies, infants with positive rWES/rWGS results were statistically more likely to have anomalies of the extremities (4/21, 19% vs. 2/59, 3%, $p = 0.04$) or multiple anomalies (10/21, 48% vs. 10/59, 17%, $p = 0.01$) compared to infants with negative findings. In the four cases of anomalies of the extremities, three were associated with other anomalies, rhizomelia (two cases) and micromelia (one case). One was an isolated unusual lower extremity finding described as bilateral feet with "puffy" anterior surface and "prominent" calves. Tables 4 and 5 show the genetic diseases detected by neonatal genomic sequencing with and without prenatal ultrasound findings, respectively. Of 14 infants with positive genomic sequencing results and prenatally detected sonographic anomalies, 7 had single gene disorders and 7 had chromosomal copy number variants or structural disorders (Table 3). Fetal growth restriction was present in 4 out of 21 (19%) infants with positive rWES/rWGS and in 4 out of 59 (7%) with negative sequencing results ($p = 0.2$).

3.3 | Potential changes in clinical management with prenatal procurement of genomic sequencing data

The 14 acutely ill infants with anomalies suspected on fetal ultrasound and positive rWES/rWGS results are detailed in Table 3. We compared the expected clinical management based on fetal phenotype alone (Column 7) and the expected change in clinical management if the genomic sequencing result was known prenatally (Column 8). In 13 cases, having the genetic diagnosis prenatally would have altered prenatal or immediate postnatal care. Additional targeted prenatal ultrasounds, prenatal consultation with pediatric subspecialists, and delivery near a level III/IV NICU were the three most common potential management changes.

One case highlights the difficulty in providing clear guidance in pregnancies complicated by minor fetal anomalies. Infant 243 was first noted to have choroid separation on prenatal ultrasound at 23 weeks' gestation. At 28 weeks, the fetus continued to have choroid separation of 4–5 mm and also developed bilateral borderline ventriculomegaly measuring 9–10 mm and polyhydramnios. These minor anomalies do not typically alarm obstetricians for significant postnatal complications or affect prenatal management. This

TABLE 1 Maternal and pregnancy outcomes for infants receiving positive and negative results from diagnostic genomic sequencing

	Positive results *N* = 21	Negative results *N* = 58	Total
Maternal age in years, mean (standard deviation)	29.8 (5.5)	30.5 (5.4)	30.3 (5.4)
Maternal ethnicity/race			
Hispanic, *n* (%)	11 (52.4%)	18 (30.5%)	29 (36.3%)
Asian, *n* (%)	2 (9.5%)	3 (5.1%)	5 (6.3%)
Pacific islander, *n* (%)	0	3 (5.1%)	3 (3.8%)
Black, *n* (%)	0	2 (3.4%)	2 (2.5%)
White, *n* (%)	10 (47.6%)	32 (54.2%)	42 (52.5%)
More than one race, *n* (%)	7 (33.3%)	14 (23.7%)	21 (26.3%)
Nulliparous, *n* (%)	8 (38.1%)	24 (40.7%)	32 (40%)
Preterm birth <37 weeks of gestation, *n* (%)	3 (14.3%)	18 (30.5%)	21 (26.3%)
Gestational age at birth in weeks, mean (standard deviation)	38.1 (2.3)	36.8 (3.9)	37.2 (3.6)
Female infant, *n* (%)	12 (57.1%)	24 (40.7%)	36 (45%)
Cesarean delivery, *n* (%)	11 (52.4%)	27 (45.8%)	38 (47.5%)
Whole genome sequencing, *n* (%)	14 (66.7%)	27 (45.8%)	41 (51.2%)

TABLE 2 Fetal anomalies detected by prenatal ultrasound in infants receiving positive and negative results from diagnostic genomic sequencing

	Positive results *N* (percentage)	Negative results *N* (percentage)	Odds ratio (95% confidence interval)	*p*[a]
Central nervous system	7 (33.3)	9 (15.3)	2.78 (0.88–8.79)	0.11
Face	2 (9.5)	0	N/A[b]	0.07
Heart	7 (33.3)	21 (35.6)	0.91 (0.32–2.59)	1
Chest	1 (4.8)	2 (3.4)	1.43 (0.12–16.6)	1
Gastrointestinal	2 (9.5)	3 (5.1)	1.97 (0.31–12.7)	0.6
Genitourinary	5 (23.8)	5 (8.5)	3.38 (0.87–13.1)	0.12
Extremities	4 (19)	2 (3.4)	6.71 (1.13–39.8)	**0.04**
Spine	1 (4.8)	0	N/A[b]	0.26
Umbilical cord	3 (14.3)	4 (6.8)	2.29 (0.47–11.2)	0.37
Placenta	1 (4.8)	0	N/A[b]	0.26
Amniotic fluid index	4 (19)	6 (10.2)	2.08 (0.52–8.25)	0.44
Other body system	0	2 (3.4)	N/A[b]	1
Any anomaly	14 (66.7)	33 (55.9)	1.58 (0.56–4.47)	0.45
Multiple anomalies	10 (47.6)	10 (16.9)	4.46 (1.49–13.3)	**0.01**
Fetal growth restriction	4 (19)	4 (6.8)	3.24 (0.73–14.3)	0.2

Note: The bolded values are statistically significant.

[a]Analysis by Fisher's exact test.

[b]Not estimable due to zero cells.

infant unfortunately was admitted to the neonatal intensive care unit (NICU) on day of life 7 in status epilepticus. He was diagnosed with pyridoxine-dependent epilepsy on day of life 9 after genomic sequencing and promptly started treatment with pyridoxine. He was discharged from the NICU on day of life 22. He is now 3 years of age and remains seizure-free on treatment. If diagnosed during pregnancy, maternal pyridoxine supplementation could have been started, and immediate therapy including dietary lysine restriction with

TABLE 3 Types of fetal anomalies detected by prenatal ultrasound in infants receiving positive results from diagnostic genomic sequencing by body system

Central nervous system	Ventriculomegaly (3)
	Dilated third ventricle
	Enlarged cavum septum pellucidum
	Microcephaly
	Choroid separation
Face	Micrognathia (2)
	Absent or hypoplastic nasal bone (2)
	Flat profile
Heart	Coarctation of the aorta or hypoplastic aortic arch (3)
	Ventricular septal defect (2)
	Atrial septal defect
	Tetralogy of Fallot
	Hypoplastic left heart
	Cardiac rhabdomyomas
Chest	Depressed shape chest
Gastrointestinal	Echogenic bowel (2)
Genitourinary	Urinary tract dilation (2)
	Echogenic kidney
	Multicystic kidney
	Absent kidney
Extremities	Short long bones (3)
	"Puffy" feet, "prominent" calves
	Talipes equinus
	Clenched hands
Spine	Abnormal spine
Umbilical cord	2 vessel cord (2)
	Hypocoiled cord

arginine and pyridoxine supplementation would have prevented neonatal seizures and avoided neonatal intensive care, which cost $66,365 in his case.

Fetal phenotype description can be imprecise. Neonate 377 was noted to have "puffy" feet and "prominent" calves on prenatal ultrasound and was postnatally diagnosed with neonatal lymphedema due to an FLT4 gene mutation. In this case, if diagnosed prenatally, serial prenatal ultrasounds to screen for hydrops and delivery near a tertiary NICU would be indicated.

Two neonates were diagnosed with 22q11.2 deletion or DiGeorge syndrome. Prior to diagnosis with DiGeorge syndrome, neonate 217 underwent cardiac catheterization for delineation of complex, structural congenital heart disease. Post-catheterization, he developed hypocalcemia, which often occurs in DiGeorge-associated hypoparathyroidism and can be associated with neonatal seizures.[13–15] Had he not been receiving care in a level IV NICU, he may not have received ongoing screening for electrolyte imbalance. Prenatal diagnosis would have prompted neonatal screening for hypocalcemia.

Some genetic disorders have signs and symptoms that vary widely. For example, neonate 302 had multiple congenital anomalies and respiratory failure and was diagnosed with 17q12 deletion syndrome, which has a highly variable phenotype.[16] In these types of cases, it is difficult to make specific prenatal or postnatal management plans, but knowledge of the genetic diagnosis could help both the obstetrical and neonatal teams anticipate and better counsel families on the range of potential complications. This neonate started palliative care shortly after birth.

There were seven infants with actionable genomic sequencing results without detected fetal anomalies. The immediate postnatal management could have been altered in six of these pregnancies (Table 5). The care for these fetuses may have been altered by their prenatal genetic diagnoses, but there would have been no ultrasound indications to seek genomic sequencing during the pregnancy.

4 | DISCUSSION

In this study we sought to explore the incidence of genetic diseases among fetuses exhibiting anomalies on ultrasound enrolled in the NSIGHT2 study. Our primary finding was that fetuses with multiple anomalies and anomalies of the extremities had a high diagnostic yield by rWES or rWGS. Notably, in 13 of 14 infants with anomalies on prenatal imaging and positive rWES or rWGS results, diagnosis in utero would have altered prenatal and immediate postnatal clinical management. In several cases, perinatal genomic medicine (management changes informed by prenatal rWES or rWGS) might have prevented long term complications and significantly reduced childhood healthcare cost by either avoiding or shortening ICU stay.

The NSIGHT2 study showed that rapid genomic sequencing benefits acutely ill infants with diseases of unknown etiology in intensive care units. Specifically, genomic sequencing changed clinical management in 28% of NSIGHT2 infants and outcomes in 15%.[8–10] Physicians caring for NSIGHT2 infants perceived that sequencing had clinical utility in 93% of infants diagnosed with single locus genetic diseases and in 72% of infants with negative results.

The data presented herein extends these findings and suggests that prenatal genetic diagnosis of single locus genetic diseases can have additional clinical utility to neonatal diagnosis. This suggests that genomic sequencing is a powerful adjunct test in the management of pregnancies complicated by fetal anomalies. Having a genetic diagnosis prenatally may have additional benefit by identification of an etiology before onset of critical illness and enabling prenatal, perinatal, and immediate postnatal interventions that decrease morbidity and mortality. Knowledge of a specific genetic disorder may, for example, alter the location (such as a facility with level III or IV NICU), timing and method of delivery, and attendant services on hand at delivery. In severe, structural, congenital heart disease,

TABLE 4 Fourteen infants with genetic diseases diagnosed by genomic sequencing and structural anomalies on prenatal ultrasound

Disease	Locus	Test	HPO term	Fetal phenotype	Fetal growth restriction[a]	Expected impact to prenatal and immediate postnatal care based on fetal phenotype	Additions to prenatal and immediate postnatal care based on genomic sequence result
HANAC (*h*ereditary *a*ngiopathy with *n*ephropathy, *a*neurysms, and muscle *c*ramps) syndrome	*COL4A2*	WES	Atrioventricular canal defect	Atrial septal defect	Yes	Neonatal echocardiogram	Serial prenatal ultrasounds screening for intracranial hemorrhage
			Abnormality of the mitral valve				Fetal MRI
			Microcephaly				Neonatal head ultrasound
			Coarctation of aorta				Delivery at level III/VI NICU
Congenital heart disease	*NOTCH1*	WES	Hypoplastic left heart	Hypoplastic left heart	No	Fetal echocardiogram	None
			Respiratory failure			Prenatal consultation with pediatric cardiology	
						Delivery at level III/VI NICU	
Pyridoxine-depending epilepsy	*ALDH7A1*	WGS	Seizures	Borderline ventriculomegaly	No	None	Fetal MRI
			Status epilepticus	Choroid separation			Maternal pyridoxine supplement
			Lactic acidosis	Polyhydramnios			Prenatal consultation with pediatric neurology
			Ventriculomegaly				Delivery at level III/VI NICU
							Neonatal pyridoxine supplement immediately after birth
Tuberous sclerosis 1	*TSC1*	WGS	Subependymal nodules	Cardiac rhabdomyomas	No	Fetal echocardiogram	None
			Predominantly lower limb lymphedema	Echogenic left kidney		Prenatal consultation with pediatric urology	
			Cardiac rhabdomyoma			Delivery at level III/VI NICU	
			Renal cyst			Neonatal echocardiogram and renal ultrasound	
Mowat-Wilson syndrome	*ZEB2*	WGS	Abnormal heart morphology	Dilated third ventricle	No	Fetal MRI	Fetal echocardiogram
			Coloboma	Bilateral urinary tract dilation		Prenatal consultation with pediatric neurology	Serial prenatal ultrasounds

TABLE 4 (Continued)

Disease	Locus	Test	HPO term	Fetal phenotype	Fetal growth restriction[a]	Expected impact to prenatal and immediate postnatal care based on fetal phenotype	Additions to prenatal and immediate postnatal care based on genomic sequence result
			Fetal pyelectasis			Neonatal renal ultrasound	Delivery at level III/VI NICU
			Low hanging columella				
Hereditary lymphedema, 1A	*FLT4*	WGS	Lymphedema	Anterior surface of feet are "puffy"	No	None	Serial prenatal ultrasounds to evaluate for hydrops
			Apnea	"Prominent" calves			Delivery at level III/VI NICU
Kabuki syndrome	*KMT2D*	WGS	Microcephaly	Microcephaly	Yes	Serial prenatal growth ultrasound	Fetal echocardiogram
			Short philtrum	2 vessel cord			Prenatal consultation with pediatric neurology
			Low set ears				Delivery at level III/VI NICU
			Generalized hypotonia				
			Small for gestational age				
			Ventricular septal defect				
			Atrial septal defect				
			Bicuspid aortic valve				
			Small nail				
22q11.2 syndrome	22q11.21 del	WGS	Tetralogy of Fallot	Tetralogy of Fallot	No	Fetal echocardiogram	Counseling on psychiatric comorbidities
						Delivery at level III/VI NICU	Chest X-ray to assess thymus and monitor calcium after birth
							Testing parents for 22q11 syndrome
22q11.2 syndrome	22q11.21 del	WES	Vomiting	Ventricular septal defect	No	Fetal echocardiogram	Counseling on psychiatric comorbidities
			Polycystic kidney dysplasia	Multicystic left kidney		Postnatal echocardiogram and renal ultrasound	Delivery at level III/VI NICU
			Ventricular septal defect	Mild polyhydramnios			Chest X-ray to assess thymus and monitor calcium after birth

(Continues)

TABLE 4 (Continued)

Disease	Locus	Test	HPO term	Fetal phenotype	Fetal growth restriction[a]	Expected impact to prenatal and immediate postnatal care based on fetal phenotype	Additions to prenatal and immediate postnatal care based on genomic sequence result
Emanuel syndrome	der(22)t(11;22)(q23;q11)	WES	Abnormal heart morphology	Absent left kidney	No	Postnatal renal ultrasound	Pediatric otolaryngologist or anesthesiologist present at birth neonatal echocardiogram and EEG
			Renal agenesis	Absent nasal bone		Delivery at level III/VI NICU	Neonatologists aware of potential gastrointestinal complications
			Micropenis	Flat profile			
			Tracheomalacia	Micrognathia			
			Pierre-Robin sequence				
17q12 deletion syndrome	17q12 del	WGS	Hydrocephalus	Bilateral ventriculomegaly	Yes	Fetal MRI	Fetal echocardiogram
			Skeletal dysplasia	Micrognathia		Prenatal consultation with pediatric orthopedic surgery	Neonatal echocardiogram
			Arthrogryposis multiplex congenita	Hypoplastic nasal bone		Delivery at level III/VI NICU	Careful glucose monitoring after birth
			Respiratory failure	Clenched hands			Neonatologists aware of potential genitourinary complications
			Hydronephrosis	Talipes equinus			
			Cytopenias	Short hypodense long bones			
				Urinary tract dilation			
				Hypocoiled cord			
				Severe polyhydramnios			
6q24-q25 deletion syndrome	6q24.2-q25.1 del	WGS	Distal ileal atresia	Ventricular septal defect	No	Fetal echocardiogram	Neonatologist aware of possible hypotonia and respiratory distress
			Respiratory failure	Hypoplastic aortic arch		Serial prenatal growth ultrasound	
			Abnormal heart morphology	Short long bones		Delivery at level III/VI NICU	
			Hypoplastic aortic arch				

TABLE 4 (Continued)

Disease	Locus	Test	HPO term	Fetal phenotype	Fetal growth restriction[a]	Expected impact to prenatal and immediate postnatal care based on fetal phenotype	Additions to prenatal and immediate postnatal care based on genomic sequence result
			Meconium ileus				
			Ventricular septal defect				
Unbalanced translocation	46XX der(22)t (19;22) (q13.42; p11.2)	WGS	Clinodactyly	Coarctation of aorta	No	Fetal echocardiogram	Offer parental karyotyping
			Coarctation of aorta	Short long bones		Serial prenatal growth ultrasound	
			Single transverse palmar crease	2 vessel cord		Delivery at level III/VI NICU	
						Neonatal echocardiogram	
19p12q13.11 duplication	19p12q13.11 dup	WES	Hematemesis	Borderline ventriculomegaly	No	None	Fetal MRI
			Gastroesophageal reflux				Prenatal consultation with pediatric neurology and gastroenterology
			Cyanotic episode				Delivery at level III/VI NICU
			Hyperbilirubinemia				Potential initiation of reflux medications immediately after birth

Abbreviations: HPO, human phenotype ontology; MRI, magnetic resonance imaging; NICU, neonatal intensive care unit; WES, whole exome sequencing; WGS, whole genome sequencing.

[a]Fetal growth restriction defined as estimated fetal weight less than 10t^{h} percentile.

TABLE 5 Seven infants with genetic diseases diagnosed by genomic sequencing and no structural anomalies on prenatal ultrasound

Disease	Locus	Test	HPO term	Fetal growth restriction[a]
Susceptible to Hirschsprung disease 1	*RET*	WES	Aganglionic megacolon Right ventricular hypertrophy Left ventricular hypertrophy	No
Prader Willi syndrome	15q11.2-q12 del	WES	Neonatal hypotonia	No
Muenke syndrome	*FGFR3*	WGS	Seizures	Yes
Transient neonatal diabetes mellitus	*ABCC8*	WGS	Hypoglycemia	No
Maple syrup urine disease	*BCKDHB*	WGS	Metabolic acidosis	No
Benign neonatal seizures 1	*KCNQ2*	WGS	Seizures	No
Early infantile epileptic encephalopathy 7	*KCNQ2*	WGS	Epileptic encephalopathy Seizures	No

Abbreviations: ED, emergency department; EEG, electroenceohalogram; HPO, human phenotype ontology; NICU, neonatal intensive care unit; WES, whole exome sequencing; WGS, whole genome sequencing.

[a]Fetal growth restriction defined as estimated fetal weight less than 10th percentile.

knowledge of the underlying genetic disorder can provide information about prognosis and complications that alters management.[17,18] In addition, diagnosis of genetic disorders in fetuses can enable implementation of precision medicine during pregnancy or immediately post birth. In one neonate, with status epilepticus due to pyridoxine dependent epilepsy, significant morbidity could have been avoided by prenatal diagnosis and precision medicine. Genetic diagnosis has implications for other family members too. For example, in the two cases of translocation, the parents could have been offered karyotype analysis. DiGeorge syndrome is inherited from a parent in 10% of cases,[19] and thus parental testing could be offered. In all cases, counseling on recurrence risk in future pregnancies and early consultation with neonatology and pediatric genetics are warranted. Another important comorbidity of DiGeorge syndrome that is often not discussed in prenatal diagnosis are psychiatric disorders such as schizophrenia later in life. Discussion beyond structural anomalies and resultant health consequences may offer a more realistic outlook on long term care.

Making a genetic diagnosis from genome sequencing is driven largely by phenotype.[7–10,20,21] The current paradigm for prenatal diagnosis is heavily dependent on ultrasound, and thus is biased toward detection of structural anomalies. Phenotypes such as seizures, hypotonia, cardiac dysrhythmias, and metabolic disorders are common reasons for neonatal illness but are challenging to detect in utero. Notably, the seven infants who had actionable rWGS/rWES results but no anomalies detected on prenatal imaging had symptoms after birth that cannot be detected by routine prenatal imaging, and six cases in this group would have had management changes if sequencing results were known prenatally. The current ability to identify cases that warrant prenatal genomic sequencing is largely limited to phenotypes that can be detected prenatally, such that expanding prenatal HPO terminology is a contemporary focus of research. Moreover, the ability to classify variants relies on prenatal phenotyping.[22]

While diagnostic test selection should be predicated on the diagnosis suggested by prenatal imaging findings, we advocate a low threshold for utilization of the much more comprehensive WES or WGS, particularly if standard testing is not diagnostic. For example, while the standard genetic test for fetal ventriculomegaly is chromosomal microarray,[23] overall diagnostic yield is only about 10%.[24] Recent innovations in WGS allow much greater analytic sensitivity for copy number variants and structural variants than chromosomal microarray.[25] We speculate that WES or WGS may have improved yield compared to microarray, both for monogenic diseases and copy number variants, in cases of fetal ventriculomegaly. Comparisons of WES versus WGS or genomic sequencing versus panel testing are beyond the scope of this study given the constraints of the study design. A critical and individualized analysis of added clinical benefit versus risks of testing such as cost, added stress, and implications to family members are important prior to clinical usage of prenatal genomic sequencing.

Prenatal phenotyping improves with targeted imaging. While three-dimensional rendering of fetal face is not routine on anatomic surveys,[26] abnormal facies or low-set ears can be visualized on ultrasound, and these additional views may be obtained when a genetic etiology is suspected.[27] Identification of subtle fetal growth disorders is also possible with personalized growth charts that consider parental ethnicity and maternal height.[28–30] We have recently found that fetal hypokinesia or akinesia may be an early sign of developmental epileptic encephalopathies.[31] However, an impediment to progress is that the prenatal phenotypes have not yet been described for many single locus genetic diseases, nor is there a full compendium of fetal phenotypes in the structured vocabularies currently used to interpret WES and WGS.

Broader adoption of fetal WES/WGS does raise clinical, logistical, and ethical concerns.[32,33] Thorough, timely interpretation of fetal WES/WGS is not widely available, as prenatal genetics remains a small field, limited to tertiary academic centers. Expansion to exome-wide and especially to genome-wide data is expected to increase risk

for uncertain test results and to prolong test turnaround time if not done via rapid genomic sequencing. These factors may lead to additional stress and frustration for both clinicians and patients.[34] Clear communication and setting expectations in pre and post-test counseling can improve the experience and foster informed decision-making.[35–37]

4.1 | Strengths and limitations

A strength of this study was that prenatal findings were described in several rare genetic disorders, adding to a relatively understudied field. More published data on prenatal presentation of these rare disorders will enrich reference databases such as Online Mendelian Inheritance in Man (OMIM) and improve data dissemination. Sharing descriptive and objective information of cases of rare genetic disorders improves our collective understanding.

This study was limited by the available prenatal imaging reports. Most infants in the NSIGHT2 study were transferred from other hospitals, and complete prenatal records were often not available. Furthermore, the level of detail and expertise in interpretation across prenatal ultrasounds varied as some ultrasounds were performed at community clinics while some were performed at fetal imaging centers. The small sample size limited the statistical power and generalizability of findings. For example, there were trends toward significant associations between neonatal rWGS/rWES findings and fetal growth restriction, facial anomalies, genitourinary tract, and central nervous system. While the odds of having a genetic diagnosis for these anomalies did not reach statistical significance, these may represent important categories of anomalies associated with pathological genetic variations that deserve further attention and research.

The study design, as a secondary analysis of another study, does limit its generalizability. We cannot calculate the diagnostic rate of a specific genetic disorder based on the specific prenatal ultrasound findings as there are infants delivered at our institution with similar suspected anomalies who were not included in NSIGHT2. We also cannot estimate the prevalence of fetuses with similar ultrasound findings who did not present critically at birth.

4.2 | Future directions

Prenatal diagnosis using WGS/WES can impact clinical management, but larger, prospective studies are needed to determine to what degree. Our findings suggest that anomalies of the face, genitourinary tract, central nervous system, and growth maybe high yield criteria for future prospective fetal sequencing studies. This study, because of its design, could not evaluate many potential benefits or harms of genomic sequencing. For example, the NSIGHT2 study showed that negative results of genomic sequencing also often have clinical utility. We are unable to explore the impact of negative genomic results. Future, prospective, large studies should examine obstetrician and parental perceptions of benefits and harms of both positive and negative results of prenatal genomic sequencing.

5 | CONCLUSION

In this study, two-thirds of infants with genetic diseases had multiple structural anomalies suspected prenatally. Prenatal genetic disease diagnosis could have altered clinical management in 13 of the 14 fetuses in whom anomalies on prenatal ultrasound were causally associated with genetic diseases. As the literature on prenatal phenotype and genetic diagnosis continues to expand to guide appropriate test selection, fetal genomic sequencing should be considered based on prenatal imaging findings. Additional studies are needed to establish the precise indications for prenatal WES/WGS and to further quantify the benefits and potential harms.

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CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

All data associated with this study are present in the paper are available at the Longitudinal Pediatric Data Resource under a data use agreement and subject to the limitations of the informed consent documents for each subject (Accession Number nbs000003.v1.p; https://www.nbstrn.org/research-tools/longitudinal-pediatric-data-resource).

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