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Novel Variant Findings and Challenges Associated With the Clinical Integration of Genomic Testing

An Interim Report of the Genomic Medicine for Ill Neonates and Infants (GEMINI) Study

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IMPORTANCE A targeted genomic sequencing platform focused on diseases presenting in the first year of life may minimize financial and ethical challenges associated with rapid whole-genomic sequencing.

OBJECTIVE To report interim variants and associated interpretations of an ongoing study comparing rapid whole-genomic sequencing with a novel targeted genomic platform composed of 1722 actionable genes targeting disorders presenting in infancy.

DESIGN, SETTING, AND PARTICIPANTS The Genomic Medicine in Ill Neonates and Infants (GEMINI) study is a prospective, multicenter clinical trial with projected enrollment of 400 patients. The study is being conducted at 6 US hospitals. Hospitalized infants younger than 1 year of age suspected of having a genetic disorder are eligible. Results of the first 113 patients enrolled are reported here. Patient recruitment began in July 2019, and the interim analysis of enrolled patients occurred from March to June 2020.

INTERVENTIONS Patient (proband) and parents (trios, when available) were tested simultaneously on both genomic platforms. Each laboratory performed its own phenotypically driven interpretation and was blinded to other results.

MAIN OUTCOMES AND MEASURES Variants were classified according to the American College of Medical Genetics and Genomics standards of pathogenic (P), likely pathogenic (LP), or variants of unknown significance (VUS). Chromosomal and structural variations were reported by rapid whole-genomic sequencing.

RESULTS Gestational age of 113 patients ranged from 23 to 40 weeks and postmenstrual age from 27 to 83 weeks. Sixty-seven patients (59%) were male. Diagnostic and/or VUS were returned for 51 patients (45%), while 62 (55%) had negative results. Results were concordant between platforms in 83 patients (73%). Thirty-seven patients (33%) were found to have a P/LP variant by 2 or both platforms and 14 (12%) had a VUS possibly related to phenotype. The median day of life at diagnosis was 22 days (range, 3-313 days). Significant alterations in clinical care occurred in 29 infants (78%) with a P/LP variant. Incidental findings were reported in 7 trios. Of 51 positive cases, 34 (67%) differed in the reported result because of technical limitations of the targeted platform, interpretation of the variant, filtering discrepancies, or multiple causes.

CONCLUSIONS AND RELEVANCE As comprehensive genetic testing becomes more routine, these data highlight the critically important variant detection capabilities of existing genomic sequencing technologies and the significant limitations that must be better understood.

Supplemental content

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An estimated 10% to 25% of neonates in neonatal intensive care units have an undiagnosed genetic disorder.[1-3] Because of the nonspecific presentation of many genetic disorders, affected neonates (1) have a 40% longer hospitalization,[4] (2) may not receive a diagnosis, (3) may be misdiagnosed, and (4) often have a prolonged diagnostic odyssey.[5] The Newborn Sequencing in Genomic Medicine and Public Health (NSIGHT) trials demonstrated the important role that rapid sequencing can have in providing a timely genetic diagnosis to improve neonatal outcome.[3,6-9] However, these platforms remain expensive and involve complex ethical dilemmas.[10] A targeted sequencing approach aimed at disorders presenting in the first year of life could limit incidental findings and reduce costs. However, the diagnostic capability of such a platform is unproven and must be compared with more standardized whole-genomic sequencing (WGS) technology prior to routine use.

The Genomic Medicine for Ill Neonates and Infants (GEMINI) Study (NCT03890679) is an ongoing, multiyear, multisite trial funded through the US National Center for Advancing Translational Science with a targeted enrollment of 400 neonates or infants younger than 1 year suspected of having an undiagnosed genetic disorder. The GEMINI Study is comparing the diagnostic yield of a novel targeted genomic sequencing platform (NewbornDx; Athena/Quest Diagnostics) with rapid WGS (rWGS). Specifically, the targeted genomic sequencing platform interrogates 1722 actionable genes for disorders that present in the first year of life. Patients and their parents undergo simultaneous testing on the targeted genomic sequencing platform and rWGS. Interpretation on both platforms is rapid (≤14 days) and targeted based on proband phenotype to establish a timely diagnosis and to avoid detecting unrelated incidental findings.

Recruitment for the GEMINI Study began in July 2019. Enrollment was exceeded by more than 30%, and 51 novel variants were identified that had never been previously reported in the literature. There was a 67% discordance between laboratories for infants found to have a diagnosis or variant possibly related to phenotype. Discordance was often caused by each laboratory's interpretation regarding the relative significance of that variant to disease presentation (ie, pathogenic [P], likely pathogenic [LP] or variant of unknown significance [VUS]). As these data emerged, it was important to share these interim findings for the benefit of undiagnosed infants with similar phenotypes and to highlight existing limitations regarding these quickly emerging technologies.

Key Points

Question Can a targeted genomic sequencing platform diagnose neonates and infants suspected of having a genetic disorder as accurately as rapid whole-genomic sequencing?

Findings In this comparative effectiveness study of 113 infants, diagnostic and/or phenotypically related variants of unknown significance were returned for 51 patients (45%), while 62 (55%) had negative results; results were concordant between platforms in 73% of patients. Of 51 positive cases, 67% differed in the reported result because of technical limitations of the targeted platform, interpretation of the variant, and/or filtering discrepancies.

Meaning The diagnostic capabilities of genomic sequencing technologies have the ability to affect clinical care but have significant limitations that must be better understood.

Methods

Parental written consent for participation in the GEMINI study was obtained with central institutional review board approval from Johns Hopkins University with approval at each participating hospital: Tufts Medical Center (Boston, Massachusetts), Rady Children's Hospital (San Diego, California), University of Pittsburgh Medical Center Children's Hospital (Pittsburgh, Pennsylvania), Mount Sinai Kravis Children's Hospital (New York, New York), North Carolina Children's Hospital (Chapel Hill), and Cincinnati Children's Hospital Medical Center (Cincinnati, Ohio). Hospitalized infants younger than 1 year with a suspected, undiagnosed genetic disorder were eligible for enrollment. Neonates were excluded if they were born at fewer than 23 weeks' gestation, had a major congenital infection, or had a genetic diagnosis that fully explained all phenotypic findings. Infants were classified as urgent if they (1) required mechanical ventilation, (2) exhibited severe neurological complications, (3) were hemodynamically unstable, or (4) were categorized as such at the request of the site's principal investigator. Urgent cases underwent ultrarapid sequencing and analysis with a preliminary report generated within 72 hours of specimen arrival.

Although trio testing was preferred, enrollment was dependent solely on the proband. Parents must opt in to receive secondary findings approved by the American College of Medical Genetics and Genomics (ACMG) for their infant and themselves.[11] Because of the phenotypic-driven interpretation, secondary findings were not sought, but rather were incidental findings of the analysis and not always detected. Secondary findings were only reported for the proband if they were (1) on the ACMG list and present in childhood or (2) they presented in childhood and there is a specific treatment available.[12] Nonpaternity is never revealed. Incest is reported to appropriate authorities for all enrolled minor mothers. In most cases, a family met with a geneticist or genetic counselor at the time of enrollment when a 3-generation pedigree was obtained to inform sequencing interpretation.

The patient provided 1 mL of whole blood in EDTA tubes for rWGS and 5 dried blood spots on filter paper (0.5 mL; Perkin Elmer; Health Sciences Spot Saver Cards; GR2261007) for the targeted genomic sequencing platform. Parents provided 3 mL of whole blood in EDTA tubes. Blood for rWGS was shipped on ice to Rady Children's Institute of Genomic Medicine; blood for the targeted genomic sequencing platform was shipped at ambient temperature to Athena/Quest Diagnostics. To facilitate rapid interpretation, human phenotype

ontology (HPO) terms were provided by the site to the laboratories for each patient.[13] HPO terms are a standardized vocabulary of phenotypic human abnormalities that accurately describe the individual being evaluated and are used to perform a targeted interrogation of the genome.[13] Pertinent demographic and clinical data were recorded. Race and ethnicity were recorded from the medical record based on parental self-reporting. Clinical utility of findings was assessed after return of results via a survey of the physician of record. Changes in clinical management, medications, surgeries, other therapies, and diagnostic testing were recorded.

rWGS Analysis and Interpretation

Clinical rWGS and ultrarapid WGS laboratories were accredited by the College of American Pathologists and certified through the Clinical Laboratory Improvement Amendments. The methods have been published in detail.[3] HPO terms were mapped to simple genetic diseases with VAAST (Fabric Genomics).[14]

Genome sequences were aligned to human genome assembly GRCh37 (hg19), and variants were identified with the DRAGEN Platform (Illumina).[14] Structural variants were identified with Manta and CNVnator and filtered to retain those coding regions of known disease genes with allele frequencies less than 2% in the Rady Children's Institute of Genomic Medicine database.[14] Nucleotide and structural variants were automatically annotated and ranked using Opal Clinical (Fabric Genomics) and manually interpreted iteratively by clinical molecular geneticists according to standard clinical guidelines.[14] Genomic sequence interpretation was performed as singleton probands. Infants undiagnosed as singletons were reanalyzed as trios.[14] If a provisional diagnosis was made with a treatment identified to prevent morbidity or mortality, it was immediately conveyed to the caregivers. Causative variants were confirmed by Sanger sequencing or chromosomal microarray.

Targeted Genomic Sequencing Platform Analysis and Interpretation

The targeted genomic sequencing platform[15] testing was performed in a College of American Pathologists-accredited, Clinical Laboratory Improvement Amendments-certified, and New York State–licensed laboratory by Athena/Quest Diagnostics. Genomic DNA was extracted using QIAmp DNA methods (Qiagen). Custom oligonucleotide probe libraries (Agilent SureSelect) captured genomic DNA regions of interest. Sequencing was performed on a NextSeq 500 (Illumina) using paired-end 75-base pair reads. Libraries were sequenced to a global mean targeted coverage of approximately 300 times and a local coverage of approximately 99% of bases 20 times or more. Sequencing reads were mapped and aligned to the reference genome GRCh37 (hg19), followed by position sorting and variant calling using Edico Dragen version 2.6.5 (Illumina). Opal Clinical software (Fabric Genomics) was used for variant interpretation and HPO-driven prioritization of causal variation. Candidate variants were assessed for pathogenicity using a standardized framework.[16] Data were gathered from multiple sources. Evidence was reviewed by a variant scientist, clinical molecular geneticist, geneticist, and genetic counselor. Plausible causal variants in genes related to phenotype were identified based on a systems approach of disease severity and body system combined with the application of phenotypically driven variant ontological reranking in the Fabric Genomics platform.[17] Assessment of variants includes inheritance pattern, frequency of variant, variant consequence, and reports in public databases. All variants were confirmed by Sanger sequencing.

Result Classification

Variants were classified as P, LP, or VUS based on HPO terms provided and each laboratory's interpretation in accordance with ACMG guidelines.[18] A VUS was only reported if located in a gene that was casually related to a genetic disease whose expected clinical features in infancy clearly overlap with the observed phenotypes in the proband. All variants were reported to ClinVar at yearly intervals per protocol. Discordant results between laboratories were defined as variant discrepancies that differed between clinically significant (P and LP) and VUS and variant discrepancies between VUS and not reported. In cases of discordance, the infant was classified into the highest level of variant classification. Analysis took place from March to June 2020.

Results

To date, 113 of the targeted 400 patients (28%) have been enrolled (eFigure in the Supplement). Pertinent clinical and demographic data of enrolled patients are listed in **Table 1**. Overall, 116 parents (79%) who were approached consented to enrollment. A total of 102 infants (90%) were analyzed as part of a duo or trio on the targeted genomic sequencing platform while rWGS reflexed to a duo or trio for 71 infants (63%). Gestational age of patients ranged from 23 to 40 weeks and postmenstrual age from 27 to 83 weeks. Sixty-seven patients (59%) were male. Enrollment per site were as follows: Tufts Medical Center, 14; Rady Children's Hospital, 27; University of Pittsburgh Medical Center Children's Hospital, 7; Mount Sinai Kravis Children's Hospital, 7; North Carolina Children's Hospital, 5; and Cincinnati Children's Hospital Medical Center, 53. Twenty-five cases (22%) were classified as urgent and underwent ultrarapid sequencing.

Diagnostic and/or VUS variants were returned for 51 patients (45%), while 62 (55%) were reported as negative. Results were concordant between platforms in 83 cases (73%). Thirty-seven patients (33%) had a P or LP variant consistent with a specific genetic diagnosis, and 14 patients (12%) had at least 1 VUS detected by 1 or both sequencing platforms (**Figure**). Patients undergoing urgent testing had 9 P/LP variants (36%) and 6 VUS (24%) (eTable 1 in the Supplement). Four infants (3%) had more than 1 diagnosis, 5 (4%) had a diagnosis and a VUS, and 2 (2%) had more than 1 VUS.

Table 1. Demographics of Study Participants

Characteristic	No. (%)
Infants, No.	113
Maternal ethnicity	
Hispanic or Latino	24 (21)
Not Hispanic or Latino	85 (75)
Not reported/unknown	4 (4)
Maternal race	
Unknown	9 (8)
Multiracial	4 (4)
American Indian	1 (1)
Asian	10 (9)
Black	20 (18)
Pacific Islander	0
White	65 (58)
Other[a]	4 (4)
Infant sex	
Female	46 (41)
Male	67 (59)
Gestational age at birth, wk	
<28	6 (5)
28-<34	15 (13)
34-<37	18 (16)
≥37	74 (65)
5-min Apgar score <5	9 (9)[b]
Postmenstrual age at time of enrollment, wk	
<28	1 (1)
28-<33	5 (4)
33-<37	10 (9)
37-<44	63 (56)
44-<48	10 (9)
≥48	24 (21)
Infant age at enrollment, d	
<30	67 (59)
31-60	13 (12)
61-90	10 (9)
91-120	5 (4)
>120	18 (16)
Urgent cases	25 (22)

[a] Parents self-reported their race as other.

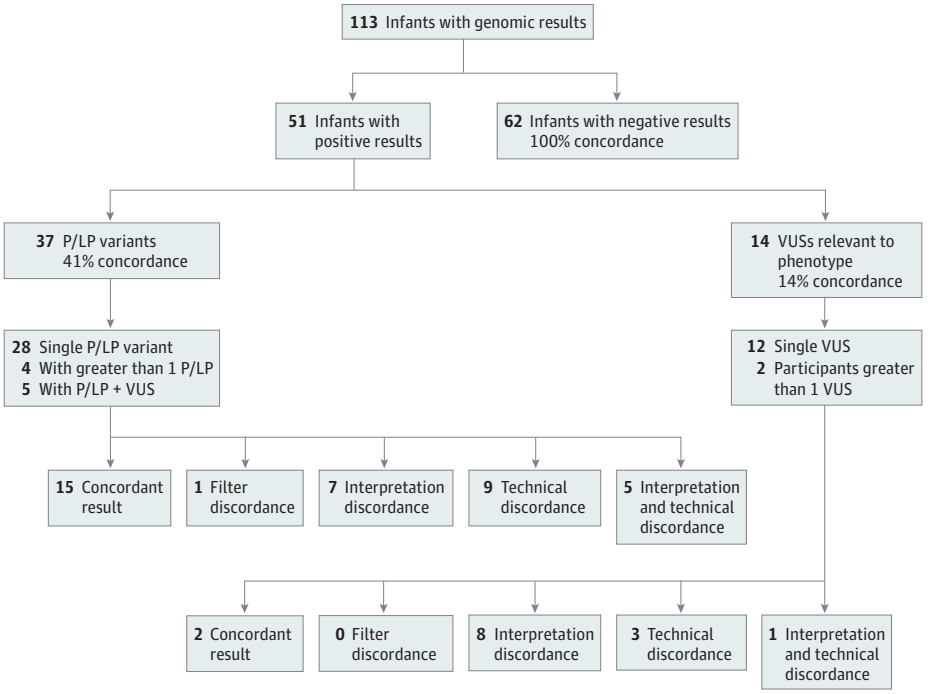
[b] Five minute Apgar score was only available for 102 infants.

A trisomy, tetrasomy, or a chromosomal deletion or duplication was identified in 9 patients (8%) by rWGS. Although 51 variants (9 P, 17 LP, and 25 VUS) have not previously been reported in the literature to our knowledge, 17 of these have been reported in ClinVar and/or gnomAD variant databases. Twelve infants (11%) had de novo variants, highlighting the significance of this inheritance pattern in this patient population. Of 51 patients with variants classified as P, LP, or VUS, results of the 2 testing platforms were discordant in 34 (67%) because of technical limitations of the platforms, variant interpretation, or both (Figure). Technical discrepancies included (1) a diagnosis of a trisomy or tetrasomy (n = 3), chromosomal duplication (n = 2), or chromosomal deletion (n = 4), which could not be detected with the targeted genomic sequencing platform; (2) the gene was not present on the targeted genomic sequencing platform (n = 8); and (3) limited coverage of the gene on the targeted genomic sequencing platform (n = 1). There was 1 computational filtering discrepancy caused by a mapping quality threshold between laboratories. Genomic results from each laboratory for infants with a diagnosis (n = 37) are provided in **Table 2** including differences in variant interpretation, technical limitations, and those that were fully concordant. eTable 2 in the Supplement includes include all remaining infants with a VUS only (n = 14). Median age at diagnosis was 22 days (range: 3-313 days); 21 infants (58%) received a diagnosis in the neonatal period (≤28 days of life). Significant changes based on a diagnosis occurred in 29 infants (29 of 37 [78%] with P/LP variant[s] detected; 29 of 113 patients [26%] tested; **Table 3**) including redirection of care from comfort to specific therapy (n = 3), redirection of care to comfort (n = 3), and/or change in medical, surgical, subspecialist, diagnostic testing, or other therapeutic management (n = 23). Of 113 parents, 105 (93%) opted to receive secondary findings for their infants, with 110 of 113 mothers (90%) and 75 of 83 fathers (90%) opting to receive their secondary findings. Secondary findings were reported among 7 trios (**Table 4**). Three parents and other family members were newly diagnosed with a genetic condition based on the infant's diagnosis.

Discussion

The GEMINI Study has provided rapid genomic sequencing results to 113 patients, identifying positive findings in 51 (45%) and a molecular diagnosis in 37 (33%). The NSIGHT trial found a 43% diagnostic rate in critically ill neonates with WGS.[7] A 2018 meta-analysis exploring the clinical utility of WGS and whole-exome sequencing in older children with suspected genetic disorders revealed a diagnostic yield of 41% and 36%, respectively.[19] Beyond independent validation, the GEMINI Study affirms the significant effect that rapid phenotypically driven genomic sequencing can have on clinical care. Although enrollment is ongoing, the GEMINI Study has already (1) directly informed clinical care in 29 of 37 newly diagnosed infants (78%); (2) diagnosed 3 parents and related family members; (3) identified 51 novel variants; and (4) identified clinically actionable secondary findings in patients and their parents. The majority of results were provided within the first 28 days of life, demonstrating a substantial reduction in time to diagnosis. With a 79% enrollment rate, the GEMINI Study reveals a strong parental desire for testing in neonates suspected of having a genetic disorder. The reasons parents declined enrollment included a fear of blaming 1 partner, belief that a neonate did not have a genetic disorder, and disinterest in pursuing genetic testing or participating in research.

Figure. Genomic Testing Results and Concordance Rates of Data Interpretation Between Laboratory Sites



LP indicates likely pathogenic; P, pathogenic; VUS, variant of unknown significance.

Importantly, results have directly informed clinical care and improved outcomes, including the identification of secondary findings. During a phenotypically driven interpretation of a trio, a *BRCA2* pathogenic variant was found in a mother unaware of her carrier status and a *RYR1* pathogenic variant was identified in a neonate at risk of malignant hyperthermia.[20] Interestingly, the GEMINI Study has identified genetic conditions in parents who have had lifelong signs/symptoms without a clear cause. One father reported a history of chorea and respiratory morbidities consistent with brain-lung-thyroid syndrome (pathogenic variant in *NKX2-1*) after his infant's testing established the genetic diagnosis.[21] It is likely that the rapid diagnostic capabilities of these testing platforms will translate into improved outcomes for both parents and their children.

Unlike many prior studies exploring the diagnostic capabilities and clinical utility of next-generation sequencing,[4-8,22,23] neonates and their parents in the GEMINI Study undergo simultaneous genetic analysis and variant interpretation on 2 distinct platforms. The challenges associated with discrepant clinical interpretation have previously been reported[24] and are caused in part by the required compilation of subjective, manual, and complex assertions that are collected from diverse sources.[18] In published comparisons, discordance in variant classification between clinical laboratory directors ranged from 12% to 71%.[24-28] The GEMINI Study further highlights the challenges of integrating this technology into care. Although there was a 73% diagnostic concordance between platforms, infants with a genomic variant had discordant reports from the 2 laboratories 67% of the time. While 56% of these discrepancies were caused by the technical limitations of the targeted genomic panel, many were due to the unique variant interpretation used by each laboratory. These data are important for 2 reasons. First, despite the use of a targeted, neonatal-specific genomic platform, some neonates will require more comprehensive genomic coverage (ie, chromosomal microarray, whole-exome sequencing, rWGS). While the targeted genomic sequencing platform is capable of detecting small copy number variants (<1000 kilobases) associated with microduplications and microdeletions, the platform currently does not leverage any copy number or structural variant detection. Second, the discrepant interpretation of variant results provided by each laboratory prompted us to report preliminary findings before study completion. Each laboratory uses the same reported phenotypes and HPO terms to direct genomic interpretation. Computational settings used to filter and rank the variants identifies some as possibly causative and can fail to identify actual contributory variants. However, despite ACMG guidelines for the interpretation and reporting of variants detected on next-generation sequencing platforms,[18] interpretation of the same variants at each laboratory also contributed to discordance. The ACMG guidelines are based on the association between reported findings in variant databases and/or the literature with their accompanying phenotypes. Therefore, by reporting our preliminary findings, along with the HPO terms that informed variant classification, we hope to improve variant detection and reporting for infants with similar phenotypes and highlight the strengths and potential limitations of these genomic platforms. This served as the primary impetus for this interim report.

The capabilities of each platform may also inform clinical interpretation. Patient 8 had 2 different P diagnoses,

Table 2. Genomic Results From Each Laboratory for Infants With a Diagnosis (n = 37)

Patient No.	Phenotype	Result[a]		Condition	Condition inheritance	Gene or No. of genes	Variant	Zygosity/ event	Variant inheritance	RCIGM classification	Athena classification	Reason for discordance	Changes in clinical management[b]
		rWGS	Targeted genomic sequencing platform										
10 Infants With Diagnostic Result(s) That Are Discordant Between Laboratories Because of Interpretation Differences[c]													
1	Thrombocytopenia, echogenic fetal bowel, single umbilical artery, atrial septal defect, ambiguous genitalia, micropenis, severe intrauterine growth restriction	U	PR	Hypogonadotropic hypogonadism 3 +/− anosmia		*PROKR2*	c.563C>T; p.Ser188Leu	Het	Mat	VUS	P	INT	
		U	NR	Duplication of 14q23.1	AD	18 Genes	chr14:59001701-61049600, dup (14q23.1)[d]	Dup	Pat	VUS	NR	SV	Yes
5[e]	Seizures, cerebellar hemorrhage, apnea, short nasal bridge, upslanted palpebral fissure, anteverted nares	PR	NR	Thrombophilia due to activated protein C resistance	AD	*F5*	c.1000A>G; p.Arg334Gly	Het	Pat	LP	NR	INT	Yes
		PR	NR	COL4A1-related disorders	AD	*COL4A1*	c.2662G>C; p.Gly888Arg	Het	de novo	P	NR	Gene	
8[e]	Bilateral cleft palate, microretrognathia, preauricular pit, redundant neck skin, hypertelorism, depressed nasal bridge, low-set ears, anteverted nares, thin upper lip vermilion	NR	PR	Lymphedema-distichiasis syndrome	AD	*FOXC2*	c.443_449dup; p.Asp151GlyfsTer314[d]	Het	Mat	NR	LP	INT	Yes
		PR	NR	Chromosome 22q11.2 duplication syndrome		58 Genes	chr22:18883701-21541000, dup (22q11.21)	Dup	Pat	P	NR	SV	
18	Dilated cardiomyopathy, cardiomegaly, atelectasis, broad nasal tip, anteverted nares	PR	U	Atrial septal defect 7 +/− AV conduction defects	AD	*NKX2-5*	c.524T>C; p.Leu175Pro[d]	Het	de novo	LP	VUS	INT	
		U	NR	Bradycardia and cardiomyopathy	AD	*HCN4*	c.2839G>A; p.Gly947Arg[d,f]	Het	Mat	VUS	NR	INT	Yes
		U	NR	Combined oxidative phosphorylation deficiency-31	AR	*MIPEP*	c.1508C>T; p.Ser503Leu[d,f]	Het	Mat	VUS	NR	INT	
		U	NR			*MIPEP*	c.590T>C; p.Leu197Pro[d,f]	Het	Pat	VUS	NR	INT	
22	Myelocystocele, neurogenic bladder, gray matter heterotopia, partial absence of the septum pellucidum, ventriculomegaly, abnormality of the cerebral white matter, fusion of the left and right thalami, micrognathia, hypertelorism, large for gestational age	PR	NR	*SPECC1L*-related disorders	AD	*SPECC1L*	c.110dup; p.Gly38ArgfsTer19[d]	Het	Not mat	LP	NR	INT	No

(*continued*)

Table 2. Genomic Results From Each Laboratory for Infants With a Diagnosis (n = 37) (continued)

Patient No.	Phenotype	Result[a]		Condition	Condition inheritance	Gene or No. of genes	Variant	Zygosity/ event	Variant inheritance	RCIGM classification	Athena classification	Reason for discordance	Changes in clinical management[b]
		rWGS	Targeted genomic sequencing platform										
28	Intrauterine growth retardation, dextrotransposition of the great arteries, perimembranous ventricular septal defect, bilateral superior vena cava with no bridging vein, dysplastic pulmonary valve, pulmonary artery dilatation, midline liver, cleft lip, wide mouth, retrognathia, micrognathia, butterfly vertebrae, sacral dimple, brain imaging abnormality, abnormal echocardiogram, recurrent fever, respiratory failure, remnants of the hyaloid vascular system, vitreous hemorrhage, stroke, seizures, thrombocytopenia, anemia, hepatitis	PR	NR	Microcephaly, short stature, and polymicrogyria +/− seizures	AR	*RTTN*	c.1008-1G>A[d]	Het	Pat	LP	NR	INT	No
		PR	NR			*RTTN*	c.3761C>T; p.Ala1254Val[d,f]	Het	Mat	VUS	NR	INT	
29	Seizures, encephalopathy, lactic acidosis, feeding difficulties, irritability, hypertonia, respiratory distress	PR	U	Neurodevelopmental disorder +/− hyperkinetic movements and seizures	AD	*GRIN1*	c.1911C>G; p.Asn637Lys[d]	Het	de novo	LP	VUS	INT	No
		U	NR	Deletion of 18q22.3		7 Genes	chr18:69444430-71765294, del (18q22.3)	del	Mat	VUS	NR	SV	
43	Severe combined immunodeficiency, immunodeficiency, lymphopenia, abnormal lymphocyte count, jaundice, umbilical vein varix	PR	NR	Coenzyme Q10 deficiency, primary 1	AR	*COQ2*	c.590G>A; p.Arg197His	Het	Mat	LP	NR	INT	Yes
		PR	NR			*COQ2*	c.151A>G; p.Met51Val[d]	Het	Pat	VUS	NR	INT	

(continued)

Table 2. Genomic Results From Each Laboratory for Infants With a Diagnosis (n = 37) (continued)

Patient No.	Phenotype	Result[a]		Condition	Condition inheritance	Gene or No. of genes	Variant	Zygosity/ event	Variant inheritance	RCIGM classification	Athena classification	Reason for discordance	Changes in clinical management[b]
		rWGS	Targeted genomic sequencing platform										
44	Intrauterine growth retardation, abnormality of movement, involuntary movements, exaggerated startle response, apnea, posterior fossa cyst, arachnoid cyst, enlarged cisterna magna, asymmetric ventricles, abnormality of the cerebral ventricles, hydronephrosis, feeding difficulties in infancy, poor suck, upper limb hypertonia, wide anterior fontanel, abnormality of cranial sutures, widely patent sagittal suture, abnormal tongue morphology, underdeveloped supraorbital ridges, periorbital edema, short palpebral fissure, depressed nasal bridge, low hanging columella, smooth philtrum, thin upper lip vermilion, abnormality of the outer ear, aplasia/hypoplasia of the external ear, maternal diabetes	PR	NR	Cornelia de Lange syndrome 5	XL	*HDAC8*	c.110G>A; p.Arg37Gln[d]	Het	de novo	LP	NR	INT	No
47	Coarctation of aorta, abnormal heart morphology, bicuspid aortic valve, hypoplastic aortic arch, left-sided atrial enlargement, mitral regurgitation, left ventricular systolic dysfunction, generalized hypotonia, abnormal corpus callosum morphology, abnormality of the periventricular white matter, gliosis, dilation of lateral ventricles, global developmental delay, torticollis, atopic dermatitis, hip dysplasia	PR	U	Kabuki syndrome 2	AD	*KDM6A*	c.3655T>C; p.Trp1219Arg[d]	Het	de novo	LP	VUS	INT	Yes

(*continued*)

Table 2. Genomic Results From Each Laboratory for Infants With a Diagnosis (n = 37) (continued)

Patient No.	Phenotype	Result[a]		Condition	Condition inheritance	Gene or No. of genes	Variant	Zygosity/ event	Variant inheritance	RCIGM classification	Athena classification	Reason for discordance	Changes in clinical management[b]
		rWGS	Targeted genomic sequencing platform										
11 Infants With Diagnostic Result(s) That Are Discordant Between Laboratories Because of Only Technical Limitations													
4	Status epilepticus, oligohydramnios,	PR	PR	Pyridoxamine 5-prime-phosphate oxidase deficiency	AR	*PNPO*	c.686G>A; p.Arg229Gln	Homo	Unk	P	P	NA	Yes
		PR	NR	16p13.11 Microdeletion syndrome		21 Genes	chr16:15124301-16788200, del (16p13.11)	Del	Unk	P	NR	SV	
6	Ventriculomegaly, cerebellar vermis hypoplasia, abnormal facial shape, hypertelorism, blepharophimosis, loss of eyelashes, depressed nasal bridge, short nasal bridge, broad nasal tip, anteverted nares, wide mouth, thin vermilion border, low-set ears, abnormality of the helix, inverted nipples, wide intermamillary distance, overlapping fingers, long fingers, micropenis, postaxial foot polydactyly, sandal gap	PR	NR	Distal trisomy 17q	NA	784 Genes	chr17:32147833-79020944, dup (17q12q25.3)	Dup	de novo	P	NR	SV	Yes
16	Hemolytic anemia, encephalomalacia, enlarged cisterna magna, gray matter heterotopia, cavum septum pellucidum, schizencephaly, cholestasis, jaundice, abnormality of the cerebral ventricles, hypoglycemia	PR	NR	Brain small vessel disease with or without ocular anomalies	NA	*COL4A1*	chr13:110863178-110864589, del (13q34)[c]	Del	de novo	LP	NR	Gene[g]	Yes
19	Pierre-robin sequence, cleft palate, micrognathia, flat face, depressed nasal bridge, almond-shaped palpebral fissure, upslanted palpebral fissure, narrow mouth, obstructive sleep apnea	PR	NR	Mental retardation 26	AD	*AUTS2*	chr7:69736006-69781006, del (7q11.22)	Del	Unk	LP	NR	Gene[g]	Yes

(*continued*)

Table 2. Genomic Results From Each Laboratory for Infants With a Diagnosis (n = 37) (continued)

Patient No.	Phenotype	Result[a]		Condition	Condition inheritance	Gene or No. of genes	Variant	Zygosity/ event	Variant inheritance	RCIGM classification	Athena classification	Reason for discordance	Changes in clinical management[b]
		rWGS	Targeted genomic sequencing platform										
20	Arthrogryposis multiplex congenita, bilateral talipes equinovarus, contractures of the joints of the lower limbs, neonatal sepsis, meningitis, communicating hydrocephalus, abnormal cardiac ventricular function, polyhydramnios	PR[h]	NR	16p13.11 Microdeletion syndrome	NA	15 Genes	chr16:15445601-18428000, del (16p13.11-16p12.3)	Del	Unk	P	NR	SV	No
30	Intrauterine growth retardation, failure to thrive, patent foramen ovale, laryngomalacia, intestinal malrotation, hydrocephalus, retrognathia, nystagmus, abnormality of eye movement, macrocephaly, Setting-sun eye phenomenon, low-set ears, convex nasal ridge, hyperpigmentation of the skin, bilateral conductive hearing impairment	PR	NR	Tetrasomy 9p	NA	365 genes	chr9:1-38834900,x4 (9p24.3p13.1)	Dup	Not mat or pat	P	NR	SV	Yes
40	Small face, short palpebral fissure, microtia, abnormal ear morphology, smooth philtrum, micrognathia, short sternum, overlapping fingers, broad hallux, abnormality of the hallux, rocker bottom foot, sacral hypertrichosis, generalized hypotonia, seizures, enlarged cisterna magna, arachnoid cyst, dysplastic tricuspid valve, abnormal mitral valve morphology, abnormal heart valve morphology, enlarged eustachian valve, polyhydramnios, maternal diabetes, 2-4 toe syndactyly, apnea, hypoplastic labia majora, enlarged labia minora, systolic heart murmur	PR	NR	Trisomy 18	NA	358 Genes	chr18:1-78077248, dup (18p11.32q.23)	Dup	Not mat or pat	P	NR	SV	Yes

(continued)

Table 2. Genomic Results From Each Laboratory for Infants With a Diagnosis (n = 37) (continued)

Patient No.	Phenotype	Result[a]		Condition	Condition inheritance	Gene or No. of genes	Variant	Zygosity/ event	Variant inheritance	RCIGM classification	Athena classification	Reason for discordance	Changes in clinical management[b]
		rWGS	Targeted genomic sequencing platform										
	Recurrent bacterial skin infections, scaling skin,	PR	U			*DOCK8*	c.1648C>T; p.Arg550Ter[d,i]	Het	Pat	LP	LP	NA	
46	recurrent *Staphylococcus aureus* infections, erythema, immunodeficiency	PR	U	Hyper-IgE recurrent infection syndrome	AR	*DOCK8*	c.*198G>A[d]	Het	Mat	VUS	NR	COV	Yes
48	Recurrent fever, anemia, thrombocytopenia, increased serum ferritin, splenomegaly, failure to thrive	PR	NR	von Willebrand disease	AD	*VWF*	c.3797C>T; p.Pro1266Leu	Het	Pat	LP	NR	FILT	Yes
	Atrial septal defect, ventricular septal defect,	PR	NR	Luscan-Lumish syndrome	AD	*SETD2*	c.5122C>T; p.Arg1708Ter[d]	Het	Pat	LP	NR	Gene[i]	
	supraventricular tachycardia, intraventricular	U	NR	NKX2-5 related disorder	AD	*NKX2-5*	c.23C>T; p.Thr8Met[d,f]	Het	Mat	VUS	NR	INT	
49	hemorrhage, poor appetite, lethargy, abnormal cry, generalized hypotonia, abnormality of the face, thin vermilion border, movement abnormality of the tongue												Yes
	Elevated plasma acylcarnitine levels,	PR	PR			*MMACHC*	c.615C>G; p.Tyr205Ter	Het	Mat	P	P	NA	
	abnormal circulating methionine concentration,	PR	PR	Methylmalonic aciduria and homocystinuria, cblC type		*MMACHC*	c.271dup; p.Arg91LysfsTer14	Het	Pat	P	P	NA	
51	abnormality of metabolism/homeostasis,				AR								No
	neutropenia, abnormality of the cerebral ventricles,	PR	NR	Deletion of 3q11.2		49 Genes	chr3:95560647-102369178 (3q11.2q12.3)[d]	Del	Mat	LP	NR	SV	
	intracranial cystic lesion, feeding difficulties, generalized hypotonia												
	16 Infants Whose Diagnostic Results Are Completely Concordant Between Laboratories												
7	Neonatal respiratory distress, respiratory failure	PR	PR	NKX2-1 related disorders; choreoathetosis and congenital hypothyroidism +/− pulmonary dysfunction	AD	*NKX2-1*	c.733A>T; p.Lys245Ter[d]	Het	Pat	LP	LP	NA	Yes

(continued)

Table 2. Genomic Results From Each Laboratory for Infants With a Diagnosis (n = 37) (continued)

Patient No.	Phenotype	Result[a]		Condition	Condition inheritance	Gene or No. of genes	Variant	Zygosity/ event	Variant inheritance	RCIGM classification	Athena classification	Reason for discordance	Changes in clinical management[b]
		rWGS	Targeted genomic sequencing platform										
9	Cephalohematoma, cerebellar hemorrhage, disseminated intravascular coagulation, hydrocephalus, abnormality of fontanelles, hyponatremia, blue sclerae, spontaneous peritoneal hemorrhage, reduced factor XIII activity, lactic acidosis	PR	PR	Deficiency of factor XIII, A subunit	AR	*F13A1*	c.27del; p.Phe9LeufsTer67	Homo	Mat and pat	P	P	NA	Yes
12	Hydrops fetalis, pleural effusion, respiratory distress, butterfly vertebrae, hepatic failure, congenital posterior urethral valve, hydronephrosis, low-set ears, anteverted nares, adrenal insufficiency, thrombocytopenia	PR	PR	Kabuki syndrome 1	AD	*KMT2D*	c.13090C>T; p.Gln4364Ter[d]	Het	de novo	P	P	NA	Yes
13	Tracheoesophageal fistula, perimembranous ventricular septal defect, patent ductus arteriosus, right ventricular hypertrophy, left superior vena cava draining to coronary sinus, left aortic arch with cervical origin of the right subclavian artery, patent foramen ovale, pulmonary arterial hypertension, ventriculomegaly, flat face, wide nasal bridge, depressed nasal bridge, prominent supraorbital ridges, infra-orbital crease, narrow mouth, thick vermilion border, abnormality of the pinna, single umbilical artery, epicanthus	PR	PR	CHARGE syndrome	AD	*CHD7*	c.4393C>T; p.Arg1465Ter	Het	de novo	P	P	NA	Yes

(*continued*)

Table 2. Genomic Results From Each Laboratory for Infants With a Diagnosis (n = 37) (continued)

Patient No.	Phenotype	Result[a]		Condition	Condition inheritance	Gene or No. of genes	Variant	Zygosity/ event	Variant inheritance	RCIGM classification	Athena classification	Reason for discordance	Changes in clinical management[b]
		rWGS	Targeted genomic sequencing platform										
17	Micrognathia, obstructive sleep apnea, penoscrotal hypospadias, cerebellar hypoplasia, delayed myelination, narrow forehead, high palate, glossoptosis, overlapping fingers, sacral dimple, feeding difficulties, anemia, enlarged CSF spares, delayed parenchymal maturation	PR	PR	Pontocerebellar hypoplasia, type 6	AR	*RARS2*	c.1582_1583dup; p.Leu528PhefsTer2[d,i]	Het	Pat	P	LP	NA	Yes
		PR	PR			*RARS2*	c.-2A>G	Het	Mat	P	VUS	INT	
		U	NR	Cerebellar ataxia, mental retardation, and dysequilibrium syndrome 2	AR	*WDR81*	c.3190C>T; p.Leu1064Phe[d]	Het	Pat	VUS	NR	INT	
		U	NR			*WDR81*	c.5557G>A; p.Val1853Ile[d,f]	Het	Mat	VUS	NR	INT	
		U	NR	Glutathione synthetase deficiency	AR	*GSS*	c.707G>A; p.Arg236Gln[d,f]	Homo	Mat and pat	VUS	NR	INT	
23	Glutaric aciduria, feeding difficulties, aspiration, failure to thrive, progressive neurologic deterioration, metabolic encephalopathy, seizures, abnormality of the optic nerve, abnormality of the cerebrospinal fluid, brain imaging abnormality, abnormal hepatic echogenicity, poor head control, developmental regression, abnormality of the basal ganglia	PR	PR	Ethylmalonic encephalopathy	AR	*ETHE1*	c.576C>A; p.Tyr192Ter[d]	Homo	Mat het; pat, NA	P	P	NA	Yes
27	Metopic synostosis, hypoplastic left side of the heart, trigonocephaly, wide nasal bridge, upslanted palpebral fissure, micrognathia, thin upper lip vermilion, polyhydramnios	PR	PR	Kabuki syndrome 1	AD	*KMT2D*	c.9265dup; p.Val3089GlyfsTer9[d,f,i]	Het	de novo	LP	P	NA	Yes
34	Hearing impairment, nystagmus, muscular hypotonia, neonatal hypotonia, abnormality of the basal ganglia, abnormal caudate nucleus morphology, abnormal globus pallidus morphology, abnormality of the internal capsule, generalized-onset seizure, generalized tonic-clonic seizures, global developmental delay, neuroblastoma	PR	PR	Combined oxidative phosphorylation deficiency-13	AR	*PNPT1*	c.337C>T; p.Pro113Ser[d]	Het	Not mat	LP	VUS	INT	Yes
		PR	PR			*PNPT1*	c.223-1G>A[d]	Het	Mat	LP	LP	NA	

(continued)

Table 2. Genomic Results From Each Laboratory for Infants With a Diagnosis (n = 37) (continued)

Patient No.	Phenotype	Result[a]		Condition	Condition inheritance	Gene or No. of genes	Variant	Zygosity/ event	Variant inheritance	RCIGM classification	Athena classification	Reason for discordance	Changes in clinical management[b]
		rWGS	Targeted genomic sequencing platform										
	Arthrogryposis multiplex congenita, camptodactyly, microretrognathia, patent ductus arteriosus, respiratory insufficiency	PR	PR			*FKBP14*	c.362dup; p.Glu122ArgfsTer7	Het	Pat	P	P	NA	
35		PR	PR	Ehlers-Danlos syndrome kyphoscoliotic type 2	AR	*FKBP14*	c.568_570del; p.Lys190del	Het	Mat	VUS	VUS	NA	Yes
	Severe lactic acidosis, congenital lactic acidosis, right ventricular dilatation, hyperammonemia, abnormal cardiac ventricular function	PR	PR			*ACAD9*	c.253C>T; p.Arg85Ter[d,f]	Het	Pat	LP	P	NA	
36		PR	PR	Mitochondrial complex I deficiency, nuclear type 20	AR	*ACAD9*	c.1552C>T; p.Arg518Cys	Het	Mat	LP	P	NA	Yes
37	Ascites, hypoalbuminemia, proteinuria	PR	PR	Nephrotic syndrome type 1	AR	*NPHS1*	c.1745_1749del; p.Lys582ArgfsTer90[d]	Het	Mat	LP	P	NA	No
		PR	PR			*NPHS1*	c.2931T>G; p.Tyr977Ter[d]	Het	Pat	LP	P	NA	
38	Hyponatremia, hyperkalemia, hyperaldosteronism	PR	PR	Pseudohypoaldosteronism type I	AD	*NR3C2*	c.2194C>T; p.Arg732Ter	Het	Pat	P	P	NA	Yes
39	Hyperammonemia, abnormal circulating acetylcarnitine concentration, abnormality of movement, feeding difficulties, encephalopathy	PR	PR	Proprionic academia	AR	*PCCB*	c.665G>C; p.Gly222Ala[d]	Het	Mat	LP	LP	NA	No
		PR	PR			*PCCB*	c.896C>T; p.Pro299Leu[d,f,i]	Het	Pat	LP	LP	NA	
41	Diarrhea, failure to thrive, weight loss, cachexia, bloody diarrhea, abdominal distention, inflammatory abnormality of the skin, skin rash, folliculitis, eczema, granuloma, delayed umbilical cord separation, abnormality of the umbilical cord, hyperglycemia, decreased thyroid-stimulating hormone level, hypothyroidism, abnormality of thyroid physiology, elevated hemoglobin A_{1c}, anti-glutamic acid decarboxylase antibody positivity, increased circulating total IgE level, breech presentation	PR	PR	Immunodysregulation, polyendocrinopathy, and enteropathy syndrome	XL	*FOXP3*	c.1010G>A; p.Arg337Gln	Het	Mat	P	P	NA	Yes

(continued)

Table 2. Genomic Results From Each Laboratory for Infants With a Diagnosis (n = 37) (continued)

Patient No.	Phenotype	Result[a]		Condition	Condition inheritance	Gene or No. of genes	Variant	Zygosity/ event	Variant inheritance	RCIGM classification	Athena classification	Reason for discordance	Changes in clinical management[b]
		rWGS	Targeted genomic sequencing platform										
42	Holoprosencephaly, alobar holoprosencephaly, seizures, hydrocephalus, severe hydrocephalus, macrocephaly, abnormal oral frenulum morphology, hypotelorism, downslanted palpebral fissures, abnormality of the nose, abnormal nasal morphology, anteverted nares, short nose, bradycardia, generalized hypotonia, respiratory failure, depressed nasal bridge	PR	PR	Holoprosencephaly 2	AD	*SIX3*	c.801_806 + 28del[d]	Het	de novo	P	P	NA	Yes
50	Hypertonia, abnormal reflex, single transverse palmar crease, global developmental delay, delayed social development, failure to thrive, intrauterine growth retardation, growth delay, weight loss, poor suck, dysphagia, abnormal localization of kidney, localized skin lesion, skin erosion, abnormality of skin morphology, micrognathia	PR	PR	COL7A1- related disorders, Epidermolysis bullosa dystrophica	AD	*COL7A1*	c.6082G>A; p.Gly2028Arg	Het	Mat	P	P	NA	Yes

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; COV, gene coverage; CSF, cerebrospinal fluid; del, deletion; dup, duplication; FILT, filtering technology; het, heterozygous; homo, homozygous; INT, interpretation; LP, likely pathogenic; mat, maternal; NA, not applicable; NR, not reported; P, pathogenic; pat, paternal; PR, primary result (variants associated with the patient's phenotype); RCIGM, Rady Children's Institute of Genomic Medicine; rWGS, rapid whole-genomic sequencing; SV, structural variant; U, uncertain result (variants possibly associated with the patient's phenotype); unk, unknown; VUS, variant unknown significance; XL, x-linked.

[a] An infant with multiple results has all results shown in the Table.

[b] A change in clinical management includes 1 or more of the following: enabled targeted treatment that may improve long-term outcomes; surgical intervention added, changed, or removed; medication added, changed, or removed; diet changed; new specialty service, imaging, test, or comorbidity screening sought; prior specialty service, imaging, test, or comorbidity screening cancelled; withdrawal of life-sustaining treatment; redirection of care from comfort to cure; or clinical monitoring or genetic testing of family members recommended.

[c] This study does not consider a variant classification difference of P v LP as a discordant result.

[d] The variant has not been previously reported in the literature; this variant was included in Genome Aggregation Database (gnomAD) or ClinVar.

[e] Patient had 2 discordant diagnoses, 1 discordance due to interpretation differences and the other due to technical limitations; both are shown in Table 2.

[f] This variant was included in Genome Aggregation Database (gnomAD).

[g] Gene not included in the targeted genomic sequencing platform.

[h] Variant was reported by another genetic test prior to study enrollment; RCIGM confirmed the finding and neither laboratory reported an additional finding.

[i] This variant was included in ClinVar.

Table 3. Selected Changes in Management Among Infants With a Diagnostic Result

Patient No.	Condition	Enabled targeted treatment that may improve long-term outcomes	Surgical intervention added, changed, or removed	Medication added, changed, or removed	Diet changed	New specialty service, imaging, test, or comorbidity screening sought	Prior specialty service, imaging, test, or comorbidity screening cancelled	Withdrawal of life-sustaining treatment	Redirection of care from comfort to cure	Clinical monitoring or genetic testing of family members recommended
1	Hypogonadotropic hypogonadism 3 +/− anosmia; duplication of 14q23.1	No	No	No	No	Yes	No	No	No	No
4	Pyridoxamine 5-prime-phosphate oxidase deficiency; 16P13.11 microdeletion syndrome	Yes	No	Yes	No	Yes	No	No	Yes	Yes
5	*COL4A1*-related disorders; thrombophilia due to activated protein C resistance	No	No	No	No	Yes	No	No	No	No
6	Distal trisomy 17q	No	No	No	No	No	No	No	No	Yes
7	*NKX2-1*-related disorders: choreoathetosis and congenital hypothyroidism +/− pulmonary dysfunction	Yes	No	Yes	No	No	No	No	No	Yes
8	Lymphedema-distichiasis syndrome; chromosome 22q11.2 duplication syndrome	Yes	Yes	No	No	No	No	No	No	Yes
9	Deficiency of factor XIII, A subunit	Yes	No	Yes	No	No	No	No	No	No
12	Kabuki syndrome 1	Yes	No	No	No	No	Yes	No	Yes	No
13	CHARGE syndrome	No	No	No	No	Yes	No	No	No	No
16	Brain small vessel disease +/− ocular anomalies	No	No	No	No	No	No	No	No	Yes
17	Pontocerebellar hypoplasia, type 6; cerebellar ataxia, mental retardation, and dysequilibrium syndrome 2; glutathione synthetase deficiency	No	No	No	No	No	No	Yes	No	No
18	Atrial septal defect 7 +/− atrioventricular conduction defects; bradycardia and cardiomyopathy; combined oxidative phosphorylation deficiency-31	No	No	No	No	No	No	No	No	Yes
19	Mental retardation 2	No	No	No	No	No	No	No	No	Yes
23	Ethylmalonic encephalopathy	No	No	Yes	Yes	Yes	No	No	No	No
27	Kabuki syndrome 1	No	No	No	No	No	No	No	No	Yes
30	Tetrasomy 9p	No	No	No	Yes	Yes	No	No	No	Yes
34	Combined oxidative phosphorylation deficiency-13	No	No	No	No	Yes	No	No	Yes	No
35	Ehlers-Danlos syndrome kyphoscoliotic type 2	No	No	No	No	Yes	No	No	No	Yes
36	Mitochondrial complex I deficiency, nuclear type 20	No	No	No	No	No	No	No	No	Yes

(continued)

Table 3. Selected Changes in Management Among Infants With a Diagnostic Result (continued)

Patient No.	Condition	Enabled targeted treatment that may improve long-term outcomes	Surgical intervention added, changed, or removed	Medication added, changed, or removed	Diet changed	New specialty service, imaging, test, or comorbidity screening sought	Prior specialty service, imaging, test, or comorbidity screening cancelled	Withdrawal of life-sustaining treatment	Redirection of care from comfort to cure	Clinical monitoring or genetic testing of family members recommended
38	Pseudohypoaldosteronism type I	Yes	No	Yes	No	No	No	No	No	No
40	Trisomy 18	No	No	No	No	No	No	Yes	No	No
41	Immunodysregulation, polyendocrinopathy, and enteropathy syndrome	Yes	No	Yes	No	Yes	No	No	No	Yes
42	Holoprosencephaly 2	No	No	No	No	No	No	Yes	No	No
43	Coenzyme Q10 deficiency, primary 1	No	No	Yes	No	Yes	No	No	No	Yes
46	Hyper-IgE recurrent infection syndrome	No	No	Yes	No	Yes	No	No	No	No
47	Kabuki syndrome 2	No	No	No	No	Yes	No	No	No	No
48	von Willebrand disease	No	No	No	No	Yes	No	No	No	No
49	Luscan-Lumish syndrome	No	No	No	No	No	No	No	No	Yes
50	*COL7A1*-related disorders: Epidermolysis bullosa dystrophica	No	No	No	No	Yes	No	No	No	No

1 detected by each platform. Based on the HPO terms, the targeted genomic sequencing platform diagnosed the infant with a maternally inherited pathogenic variant in *FOXC2*. On further discussion, it was determined that 6 family members, including the mother, had symptoms consistent with the *FOXC2* variant, which causes lymphedema-distichiasis syndrome.[29] Conversely, despite detecting the *FOXC2* variant, Rady Children's Institute of Genomic Medicine determined that a paternally inherited 22q11.2 duplication (58 genes) in the same neonate was solely responsible for the infant's phenotype of cleft palate, microretrognathia, and dysmorphic facies.[30] The infant's diagnosis is a result of both genetic findings, made possible by simultaneously running both platforms. This demonstrates the need for more structured reporting guidelines as infants may present with more than 1 genetic disorder.

Although all patients have physical, clinical, and/or metabolic signs/symptoms highly suggestive of a genetic disorder, a specific cause was not identified for the majority of patients enrolled. The inability to diagnose patients is likely multifactorial. A rapid, phenotypically driven genome interpretation limits examination of the entire genome. Only genes known to be associated with clinical findings are interrogated on both platforms. Additionally, most genetic information obtained through WGS is not analyzed. Thus, it is possible that infants may actually have a genetic disorder that will be identified as analytical techniques and variant databases become more robust and/or the neonate develops additional phenotypic findings. It is also likely that some infants do not have a genetic cause for their clinical presentation. Teratogens and environmental exposures during key periods in gestation and/or epigenetic modifications may be contributory. Finally, the results remind us of our narrow understanding of the genome, including the role that introns likely play in genetic disease. Although technology is no longer a barrier to rapid genomic sequencing, we remain limited by our understanding and interpretation of these complex biologic processes.

Limitations

This study is limited, in part, by its targeted, phenotypic-driven analysis based on our current understanding of the human genome. By only interrogating areas of the genome known to result in the patient's presenting symptoms, a rapid return of results may be provided at the expense of a diagnosis.

Conclusions

Preliminary results of the GEMINI Study revealed that 51 of 113 infants (45%) had an important genetic variant detected. Fifty-one variants were novel and previously unpublished. While there was an overall 73% concordance between platforms for patients tested, of those with a positive finding, 67% received discordant results from the different methods. By testing 2 platforms simultaneously, GEMINI has highlighted the need for rapid dissemination of findings to better inform the field about novel variants and highlighted the existing variability in genomic sequencing technologies.

Table 4. Incidental Findings Returned

Patient No.	Testing platform	Condition	Condition inheritance	Gene	Variant	Zygosity	Variant classification
9	rWGS	Malignant hyperthermia susceptibility	AD	*RYR1*	c.1840C>T; p.Arg614Cys	Het	P
13	rWGS	Bardet-Biedl syndrome 8	AR	*TTC8*	c.1029_1032dup; p.Ala345AsnfsTer16[a]	Het	LP
				TTC8	c.271G>A; p.Gly91Arg[a]	Het	VUS
38	rWGS	Anemia, nonspherocytic hemolytic due to G6PD deficiency	AR	*G6PD*	c.292G>A; p.Val98Met	Hemi	P
				G6PD	c.466A>G; p.Asn156Asp	Hemi	VUS
40	rWGS	Hereditary breast and ovarian cancer syndrome[b]	AD	*BRCA2*	c.6486_6489del; p.Lys2162AsnfsTers	Het	P
52	Targeted genomic sequencing platform	Fabry disease	XL	*GLA*	c.1103C>T; p.Ala368Val[a]	Hemi	VUS
53	rWGS	Arrhythmogenic right ventricular dysplasia, familial, 10	AD	*DSG2*	c.523 + 1G>C[a,c,d]	Het	P
54	rWGS	Anemia, nonspherocytic hemolytic due to *G6PD* deficiency	AR	*G6PD*	c.292G>A; p.Val98Met	Het	P
				G6PD	c.466A>G; p.Asn156Asp	Homo	VUS
	Targeted genomic sequencing platform	Glycogen storage disease II	AR	*GAA*	c.922C>T; p.His308Tyr[a,d]	Het	VUS
				GAA	c.*187_203del17InsGGG[a]	Het	VUS

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; del, deletion; dup, duplication; hemi, hemizygous; het, heterozygous; homo, homozygous; LP, likely pathogenic; P, pathogenic; rWGS, rapid whole-genomic sequencing; VUS, variant unknown significance; XL, x-linked.

[a] The variant has not been previously reported in the literature; this variant was included in Genome Aggregation Database (gnomAD) or ClinVar.

[b] This variant was included in Genome Aggregation Database (gnomAD).

[c] Reported for parent.

[d] This variant was included in ClinVar.

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