Study Title:	**Genomic Medicine for Ill Neonates and Infants (The GEMINI Study)**
Grant Title:	Precision Medicine in the Diagnosis of Genetic Disorders in Neonates (U01TR002271)
NCT Identified Number:	NCT03890679
Co-Principal Investigators:	Jonathan M. Davis, MD The Floating Hospital for Children at Tufts Medical Center Tel: 617-636-5322 E-mail: jdavis@tuftsmedicalcenter.org Jill Maron, MD, MPH The Floating Hospital for Children at Tufts Medical Center Tel: 617-636-0766 E-mail: jmaron@tuftsmedicalcenter.org Pager: 617-647-2971
Project Manager:	Anne Kurfiss, MPH The Floating Hospital for Children at Tufts Medical Center Tel: 617-636-7134 E-mail: akurfiss@tuftsmedicalcenter.org
Funded by:	National Center for Advancing Translational Sciences (NCATS)
Protocol Version:	2.0, 05DEC2019

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with the United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46).

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of the protocol, consent form, and Data Safety Monitoring Plan (DSMP) must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented.

_____
Printed Name

_____ __ __ / __ __ __ / __ __
Signature Date

TERMINOLOGY

Term	Definition as used in this protocol
Infant	The research subject and proband with signs/symptoms consistent with a possible genetic disorder; in most cases the infant will likely be a neonate, but not necessarily
Guardian/parent	The person who has authority to legally consent for the infant to participate in this research, this person may or may not be a biological parent.
Biological parent(s), biological mother or biological father	Who is thought to be the true parent of the infant and may provide a blood sample for genetic sequencing and trio analysis; biological parents participating in this study are also considered research subjects and may withdraw their consent at any time.
Enrolling Site	The hospital at which the infant is enrolled into the study.
Laboratories	Refers to Athena Diagnostics, a wholly-owned subsidiary of Quest Diagnostics; Rady Children's Institute for Genomic Medicine – Clinical Genome Center (RCIGM-CGC); and Sema4 which are conducting the genetic sequencing and/or confirmatory tests.
Positive Result	Diagnostic findings related to phenotype - pathogenic variant(s) in genes interpreted to be responsible for, or contributing to, the infant's phenotype (variant classification of pathogenic or likely pathogenic).
Negative Result	The result that pathogenic variants associated with the infant's clinical phenotype were not detected.
Variant of Unknown Significance (VUS)	A variant for which the clinical significance is unknown. The variant detected may or may not explain the infant's current clinical symptoms.
Suspicious VUS	A VUS where the phenotypic fit is very good or functional confirmatory tests are readily available or the results are actionable.
Medically Actionable	There is a preventative measure or a treatment available to cure or ameliorate symptoms and improve outcome.
Secondary / Incidental Findings	Pathogenic or likely pathogenic mutations listed in the ACMG 59 or in other genes unrelated to the phenotype. Secondary findings are not intentionally sought in this protocol. The RCIGM laboratory report will use the term incidental findings in order to emphasize that a targeted analysis for secondary findings is not done. The ICF will use the term additional results.
Provisional result	A positive result that has not been confirmed by another method.
Final Result	Either a positive result or VUS that is confirmed by another method, often Sanger sequencing/qPCR, or a negative result.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: Genomic Medicine for Ill Neonates and Infants
(The GEMINI Study)

Study Intervention: NewbornDx Sequencing Evaluation (NewbornDx), Rapid Whole Genome Sequencing (rWGS)

Study Description: This multicenter, prospective clinical trial will examine the diagnostic yield and clinical utility of NewbornDx and rWGS testing in high-risk infants with signs/symptoms consistent with a possible genetic disorder. Infants will undergo both NewbornDx and rWGS testing in parallel. Genetic sequences via NewbornDx and rWGS will be generated for the infant (proband) and when available, the biological parents, and analyzed. NewbornDx and rWGS test results will be returned to the infant's clinician, medical record and parent/guardian. Those infants analyzed as a duo or trio will have a retrospective analysis done of only the infant's data to determine if the same result would have been obtained with and without the parent samples. Clinical utility will be measured by clinician opinion and changes in the infant's care as a result of the genetic sequencing test results. Quality of life (QoL) and medical resource utilization by parent survey will be collected until the infant is one year corrected gestational age. These data, along with a retrospective chart review of infants with suspected genetic disorders, will be used to understand 1-year cost and health outcomes that would have been incurred in the absence of the advanced testing. The resulting data from the trial will be used in the economic evaluation comparing NewbornDx, rWGS, and SOC over a 1-year period and used as basis to simulate the lifetime cost-effectiveness of these testing strategies. A web-based clinical reference database to provide references, clinical management guidelines, opportunities for clinical trial participation, and support groups for each condition will be developed with separate interfaces for the parent/guardian(s) and medical provider. The clinical reference database will be qualitatively assessed by a survey of medical providers.

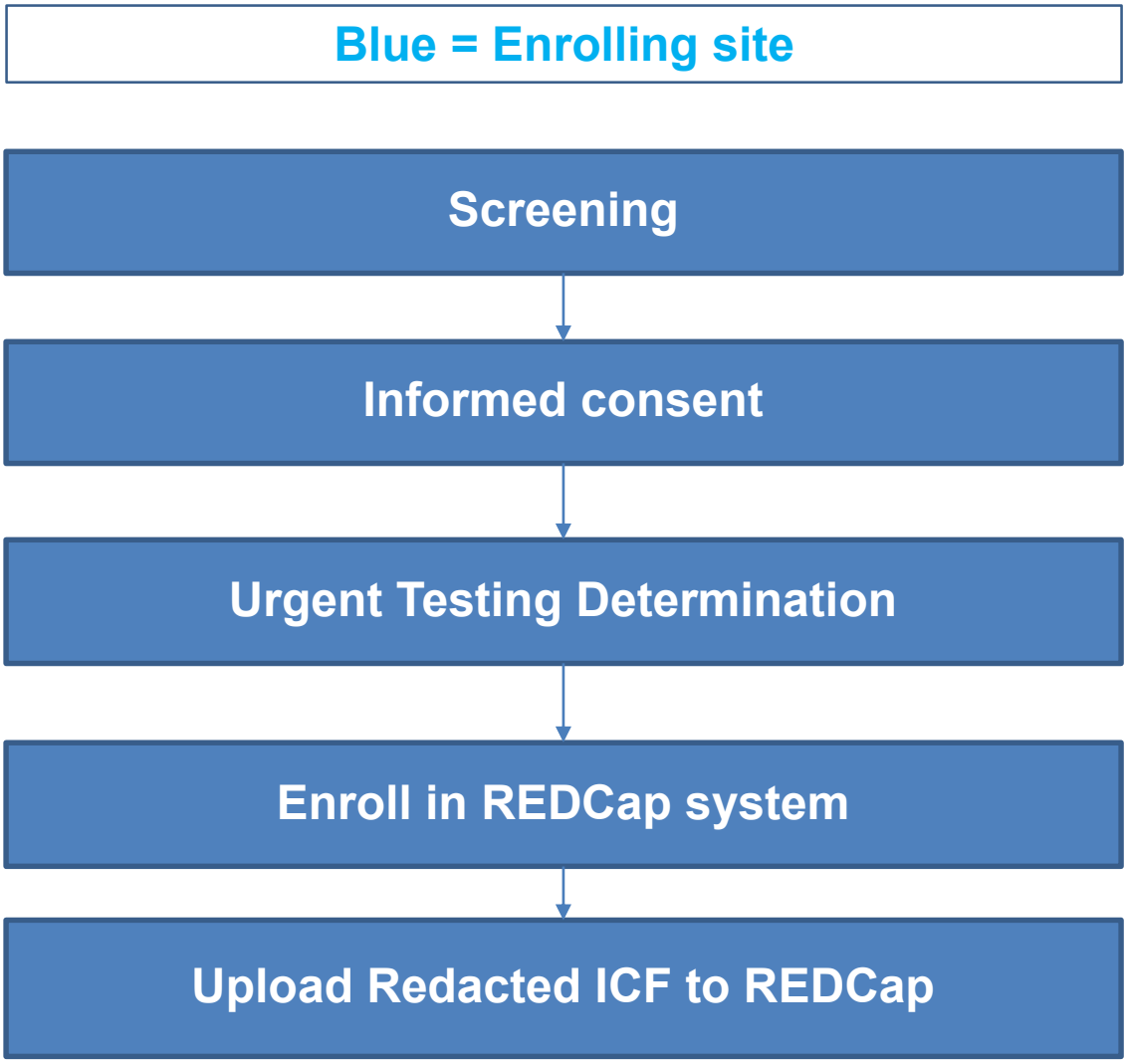
Objectives:

- To estimate the diagnostic yield of NewbornDx and rWGS testing in identifying genetic disorders of unknown etiology
- To assess the clinical utility of genomic sequencing

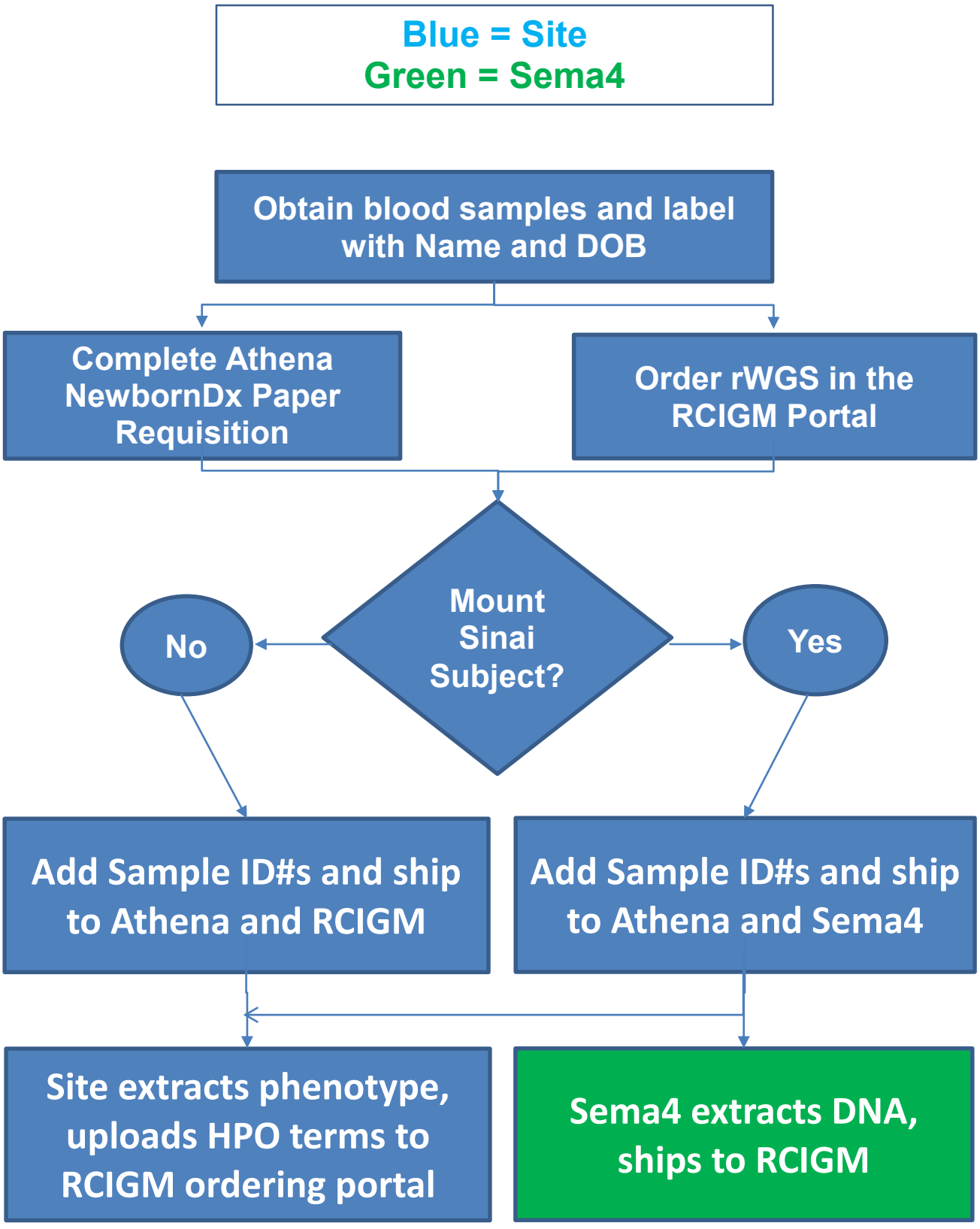
	• To examine the economic impact and health outcomes of NewbornDx and rWGS in infants compared with SOC diagnostic testing, over a one-year and lifetime horizon • To develop and qualitatively assess the web-based clinical reference database of supporting information for medical providers and parents
Endpoints:	Primary Endpoints: • A confirmed genetic disorder detected by NewbornDx • A confirmed genetic disorder detected by rWGS • Time from sample collection to positive test result • Clinical utility of genomic sequencing (care changes, time to initiation of appropriate treatment or redirection of care including the withholding or withdrawing of life-sustaining treatment) Secondary Endpoints: • One-year cost-effectiveness of SOC, NewbornDx and rWGS testing • Lifetime cost-effectiveness of SOC, NewbornDx and rWGS testing • User satisfaction with the clinical reference database
Study Population:	400 infants less than one year corrected gestational age (CGA) admitted to a hospital (NICU/PICU/ CICU/inpatient floor) participating in this study with signs/symptoms consistent with a possible genetic disorder
Phase:	N/A
Enrolling Sites:	The Floating Hospital for Children at Tufts Medical Center, Cincinnati Children's Hospital Medical Center, Mount Sinai - Kravis Children's Hospital, Children's Hospital of Pittsburgh, Rady Children's Hospital - San Diego, and North Carolina Children's Hospital
Study Duration:	54 months
Participant Duration:	From enrollment until 1 year CGA

1.2 SCHEMA

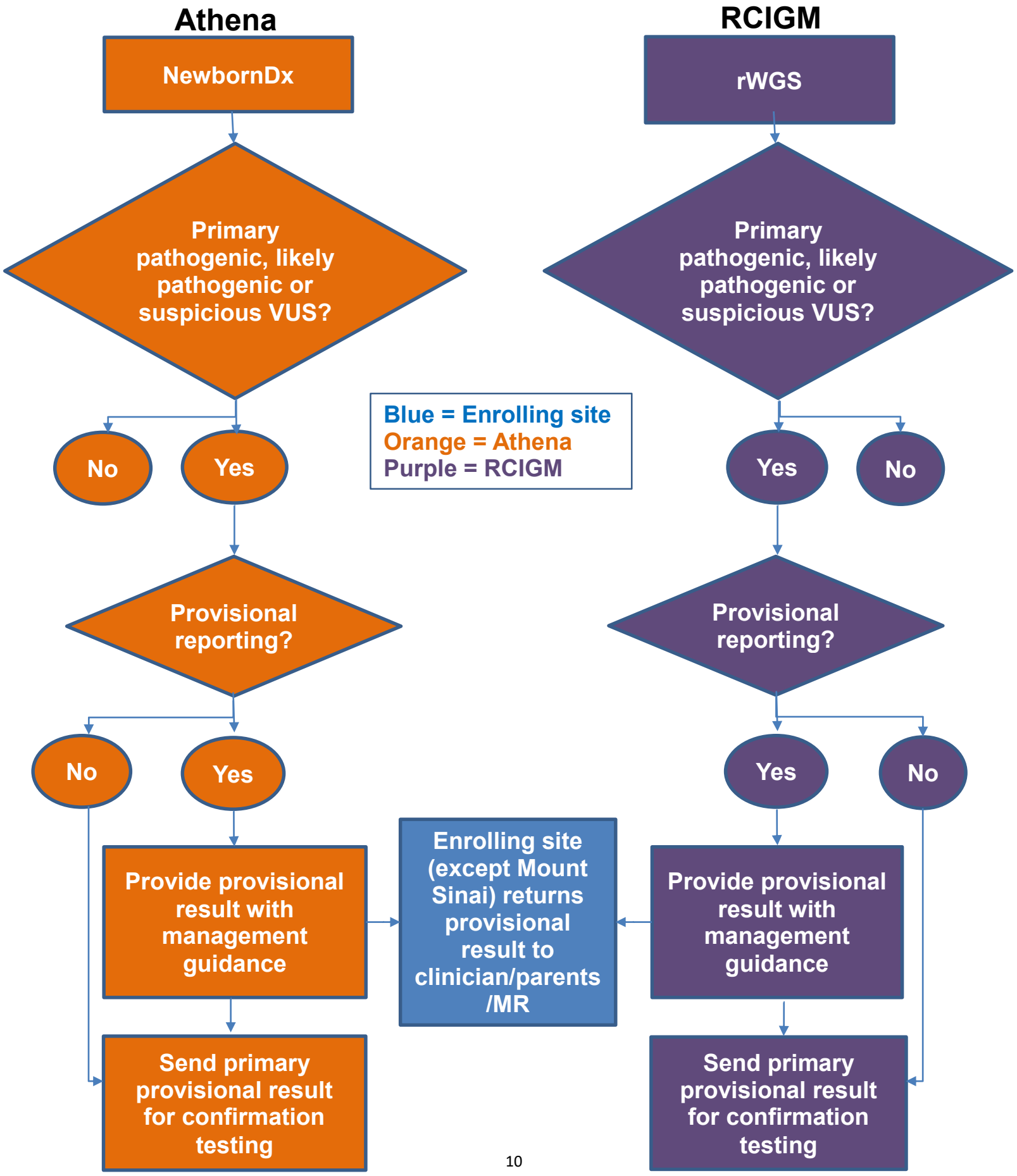
Part 1: Screening and Enrollment



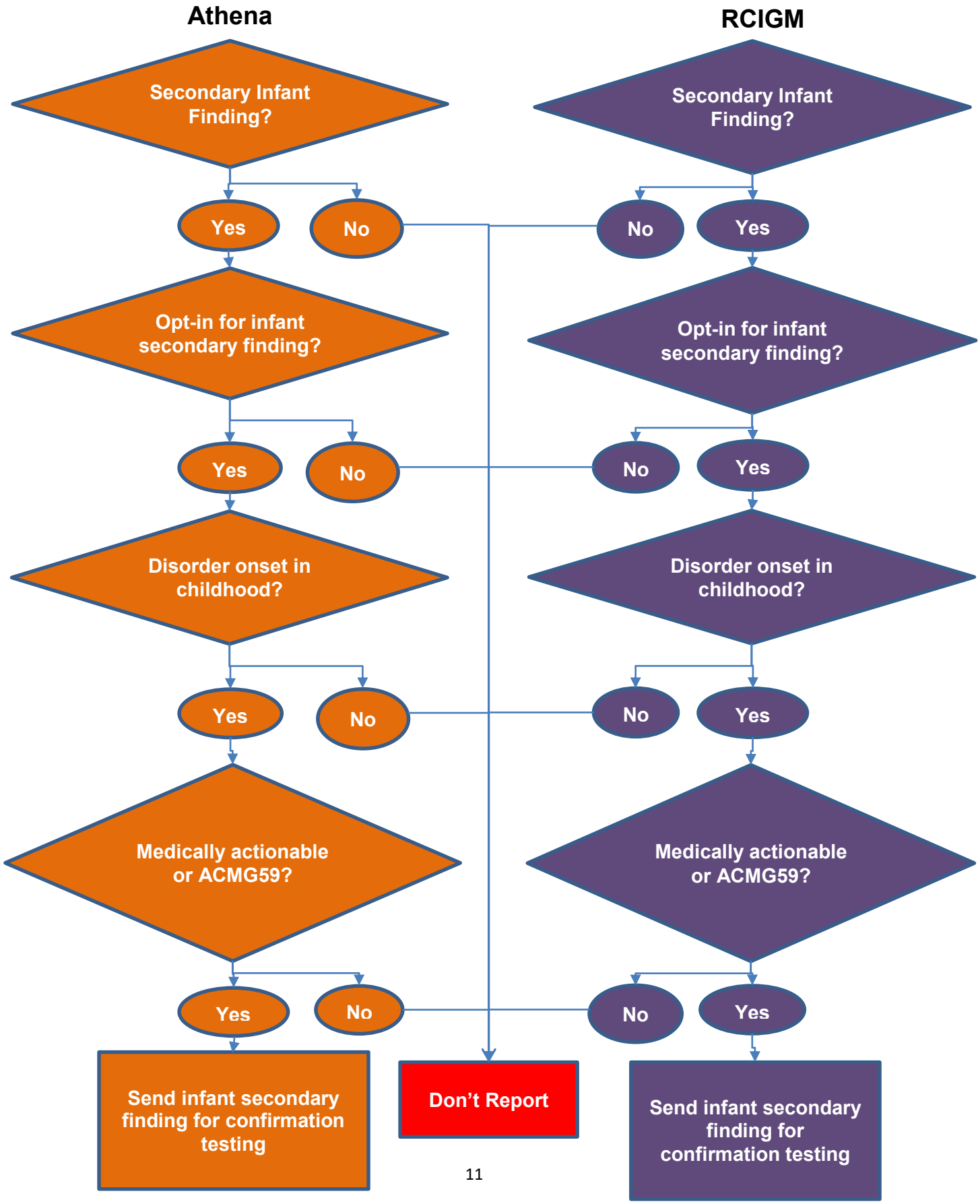
Part 2: Sample Collection and HPO



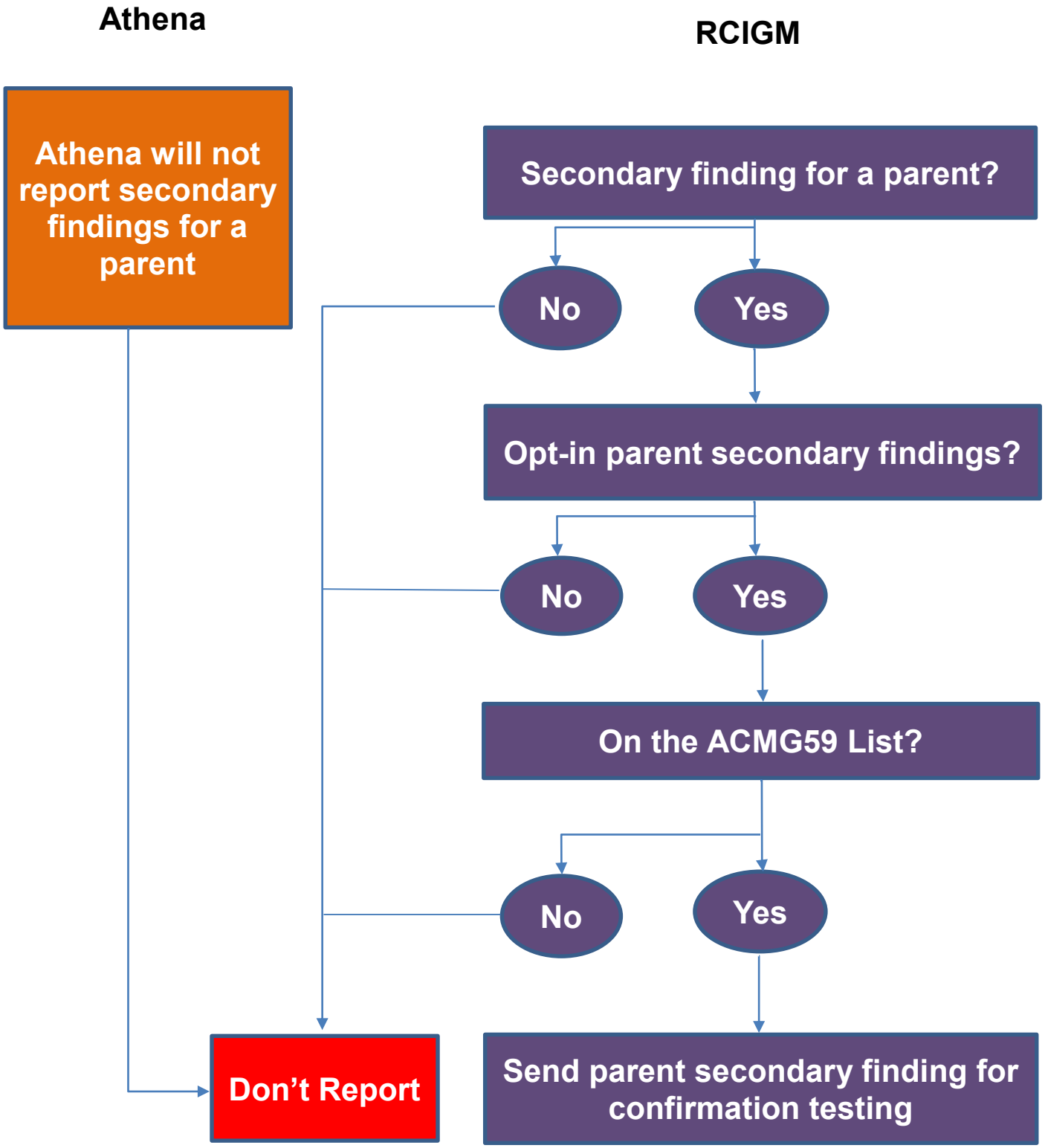
Part 3: NewbornDx/rWGS Tests



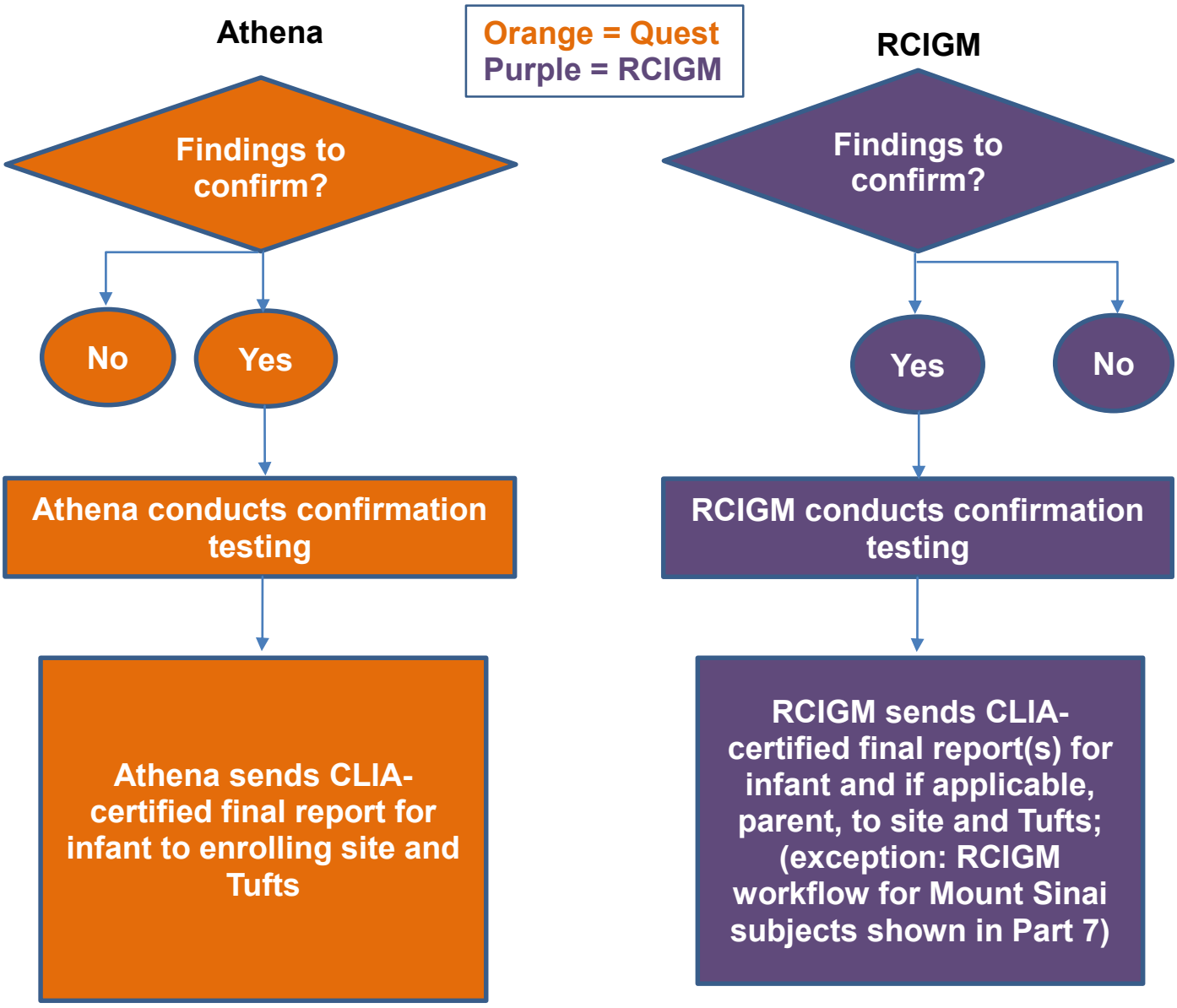
Part 4: Secondary Findings for the Infant



Part 5: Secondary Findings for a Biological Parent

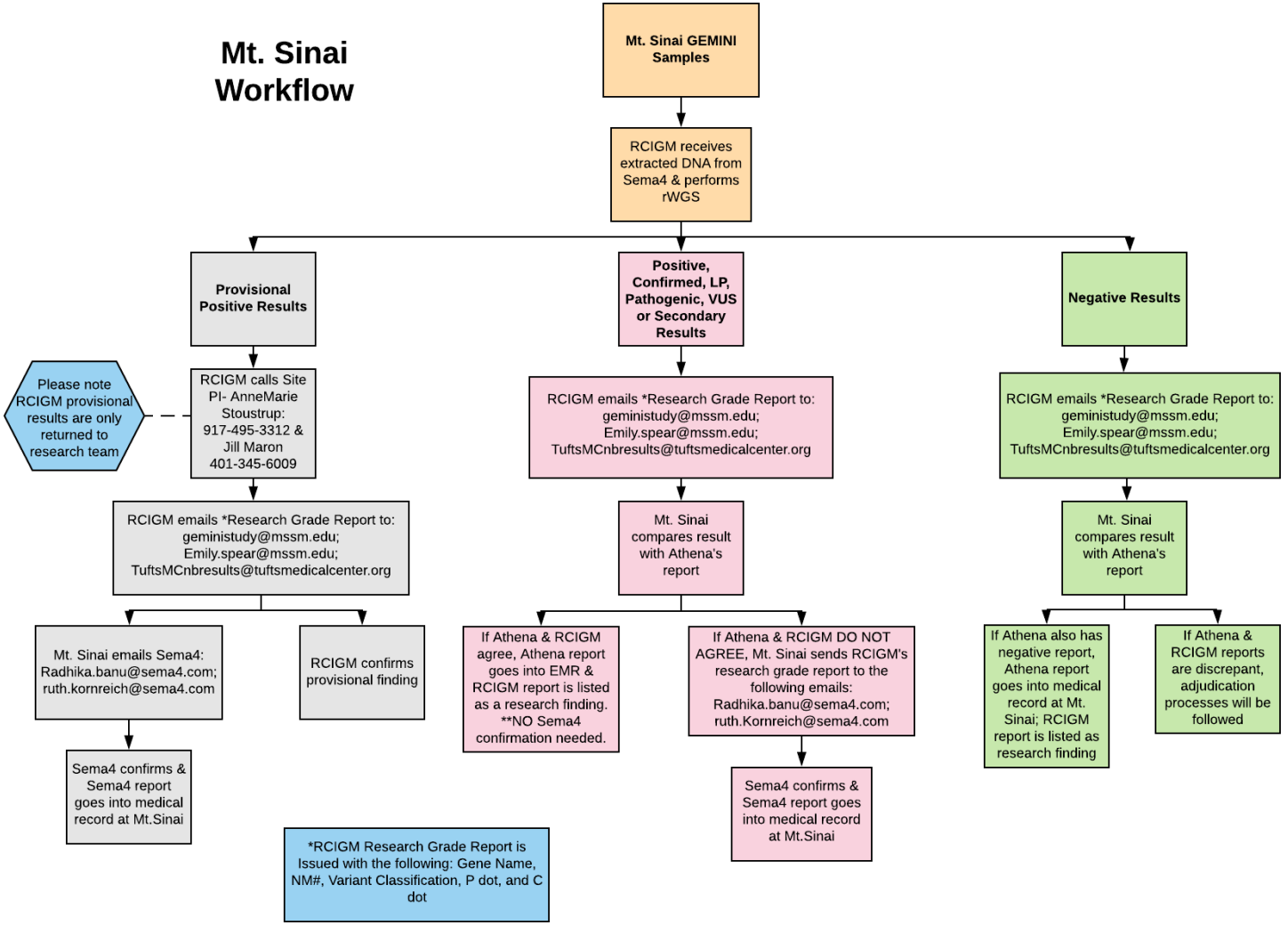


Part 6: Confirmation Testing & Return of Results



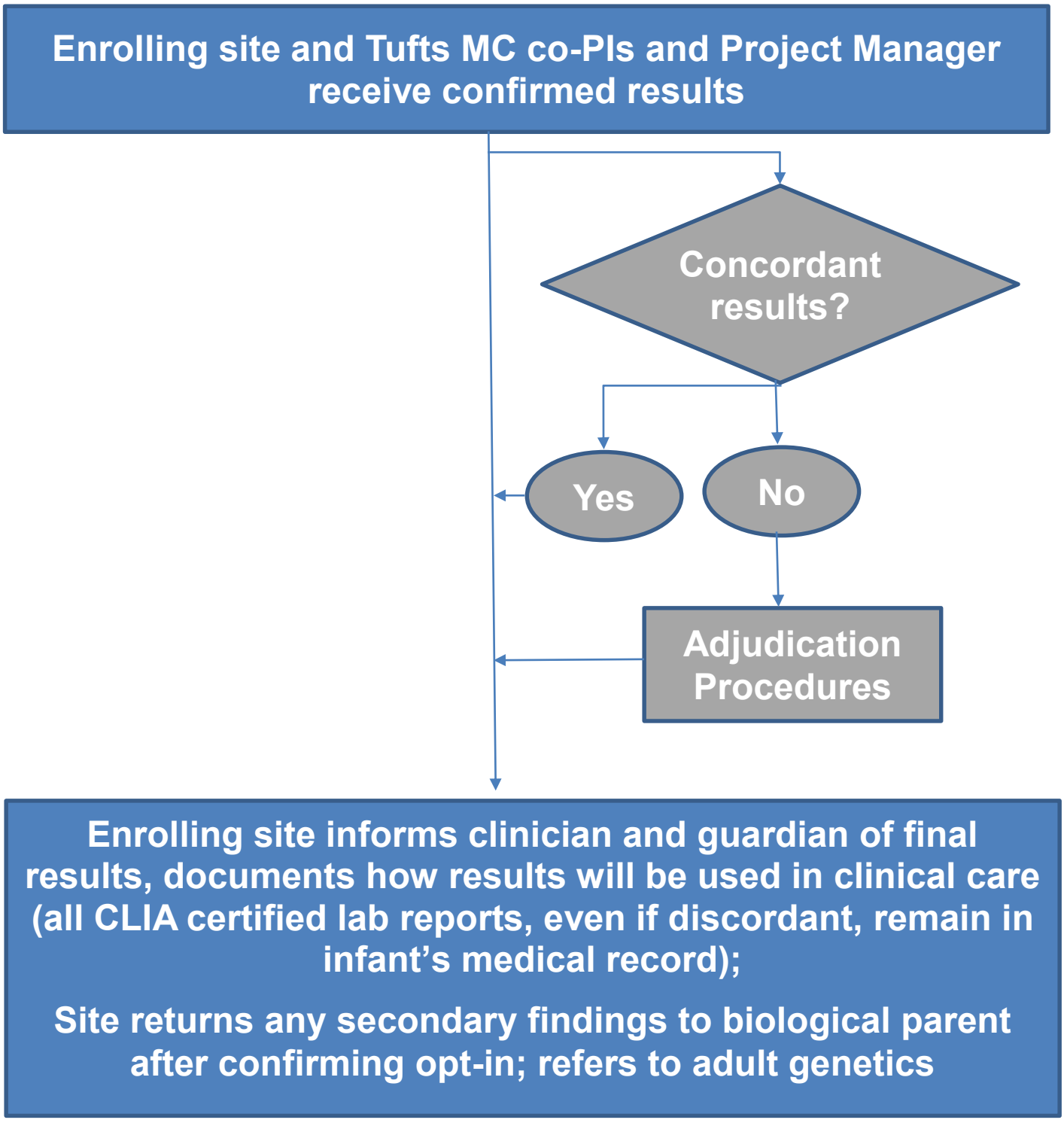
Part 7: RCIGM Workflow for Subjects from Mount Sinai Only. All other sites proceed to Part 8.

This workflow will be eliminated when rWGS becomes New York State approved

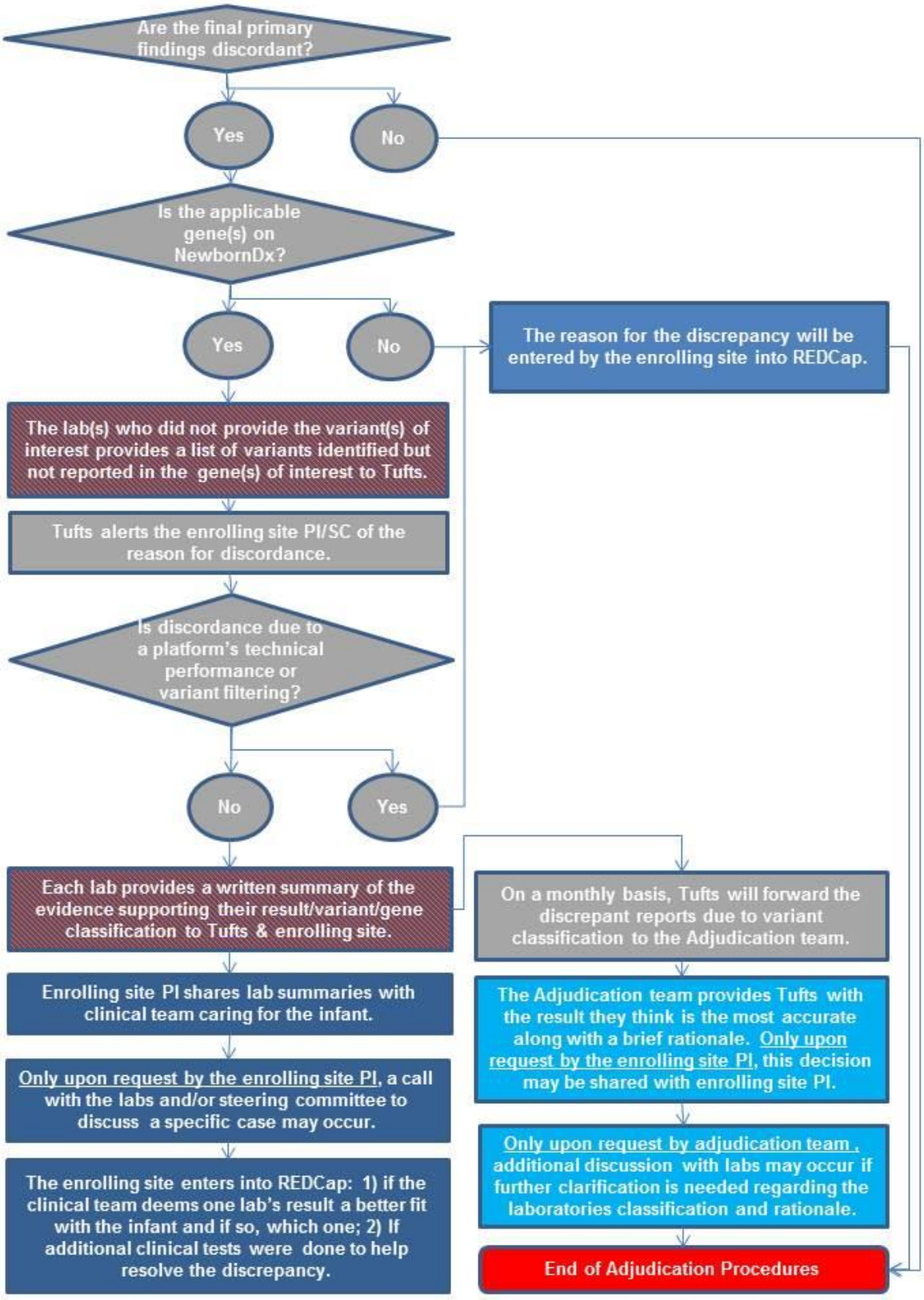


Study Workflow Part 8 - Reconciliation of Results

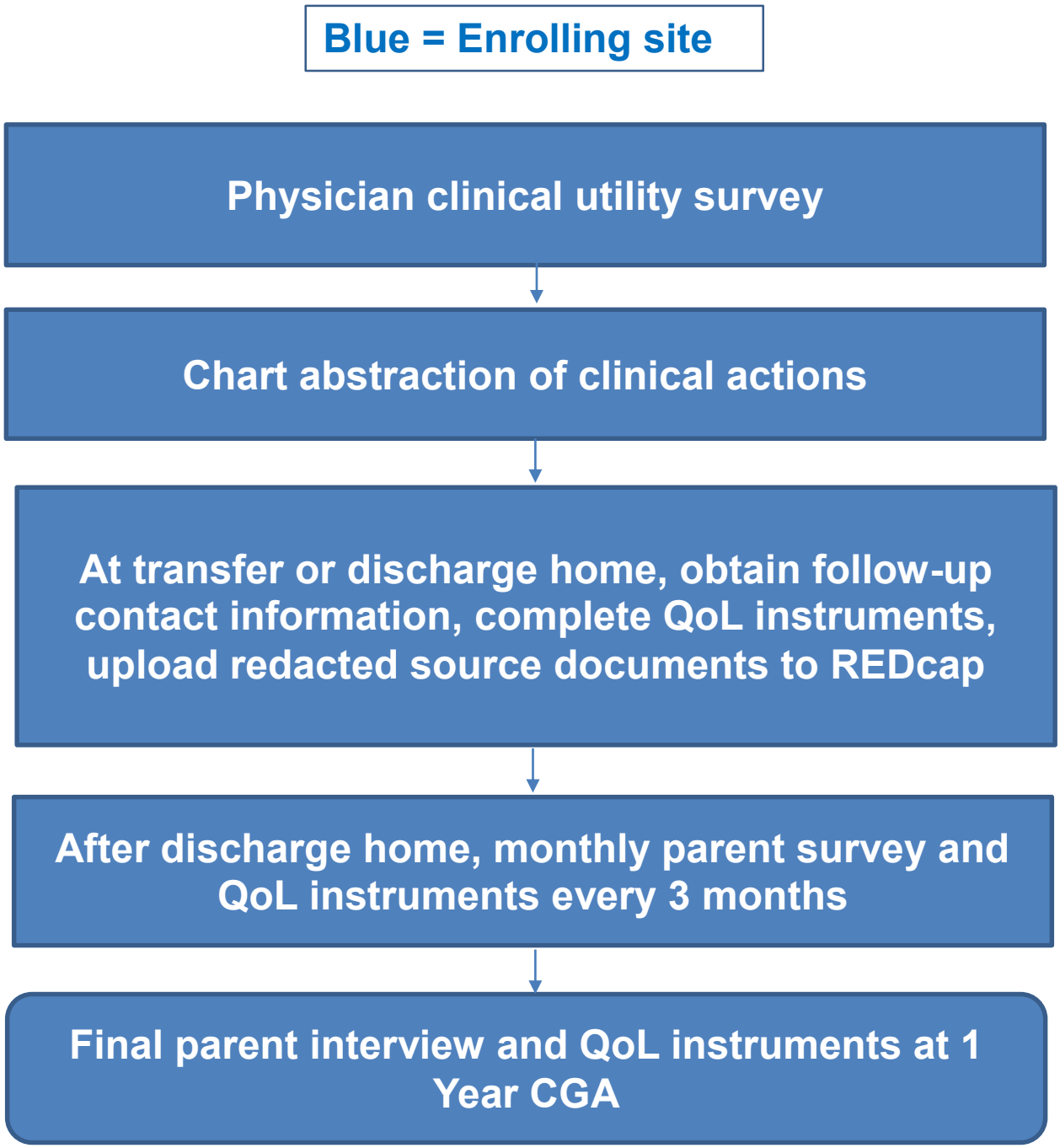
Blue = Enrolling site
Gray = Tufts



Study Workflow Part 9 – Adjudication Procedures



Part 9: Post Result through 1 Year CGA



Schedule of Activities (SoA) and Data Case Report Forms

A= Athena (Quest), R=RCIGM, S4=Sema4	Procedures	Screening	Enrollment	Genetic Sequencing	Return of final result	Transfer to facility	Discharge home	Follow-up #1-11	1 year CGA
Site	Evaluate inclusion/exclusion criteria	X							
Site	Consult clinical genetics or specialty service per SOC	X							
Site	Informed consent process	X							
Site	Complete screening log	X							
Consented and Enrolled Infants									
Site	Enroll infant in REDCap		X						
Site	Determine if the infant requires urgent testing		X						
Site	Obtain blood samples for infant and biological parent(s)		X						
Site	Order rWGS (or Ultra Rapid for Urgent Cases) in the RCIGM portal; indicate secondary finding elections for infant and each biological parent		X						
Site	Complete Athena Laboratory requisition for NewbornDx, indicate infant secondary finding election		X						
Site	Notify Athena of need for urgent testing, if applicable		X						
Site	Ship samples to Athena Diagnostics and RCIGM (or Sema4*)		X						
Site	Scan redacted ICF signature pages into REDCap		X				X		X
Site	Provide clinical information and HPO terms to RCIGM portal and Athena			X					
A	Complete NewbornDx [duo/trio if parent sample(s) are available]			X					
R	Complete rWGS [duo/trio if parent sample(s) are available]			X					
A, R	Return verbal provisional result, if applicable, with management guidance to enrolling site clinical staff*, site PI, study-wide Co-PI			X					
Site	Enter provisional result, if applicable, with management guidance as a research note to infant's medical record and notify the infant's parent/guardians			X					
A, R, S4	Complete confirmation testing for pathogenic, likely pathogenic and suspicious VUS findings*			X					
A, R, S4	Return final infant result to enrolling site research team and study-wide co-PIs/project manager				X				

A= Athena (Quest), R=RCIGM, S4=Sema4	Procedures	Screening	Enrollment	Genetic Sequencing	Return of final result	Transfer to facility	Discharge home	Follow-up #1-11	1 year CGA
R, S4	Return confirmed parental secondary findings to the enrolling site if applicable and the parent opted in on the ICF*				X				
Site	Return the final result for the infant to the clinical team, infant's medical record and the infant's parent/guardians				X				
Site	Notify the biological parent of confirmed secondary findings, if applicable and that parent opted in on ICF				X				
Tufts, Site	Follow adjudication procedures if the final results from NewbornDx and rWGS are discordant or if Sema4 does not confirm the rWGS result				X				
Site	Administer clinical utility survey				X				
Site	Obtain hospital bill(s) for infant hospitalization during which the research testing occurred					X	X		
Site	Obtain the parent/guardian contact information for follow-up					X	X		
Site	Administer parent SF-12 QoL and child visual analog scale						X	X	X
Site	Administer or if completed by email, review the parent/guardian Resource Utilization monthly interview					X	X	X	X
Site	Obtain medical records as necessary (i.e. a new diagnosis or adverse event)						X	X	X
A, R, S4	Monitor for non-conforming events or errors			X	X				
Site	Monitor for adverse events		X	X	X	X	X	X	X
Site	Monitor for protocol deviations		X	X	X	X	X	X	X

*Mount Sinai will send blood samples for the infant and each biological parent to Sema4 instead of RCIGM. Sema4 will extract the DNA, save one aliquot for future confirmation testing and ship one aliquot to RCIGM. Test results that are reported by RCIGM rWGS will be treated as research results for subjects from Mount Sinai. Results from RCIGM rWGS will be placed in the infant's research file and not the infant's medical record. Positive and VUS results reported by RCIGM that are not also reported by Athena Diagnostics will be confirmed by Sema4 and a Sema4 laboratory report will be placed in the infant's medical record. The protocol allows for the return of a provisional result when a treatment is available and waiting for confirmation testing puts the infant at irreversible harm. Provisional results will not be given for subjects from Mount Sinai when the provisional result is found only by RCIGM rWGS. A provisional result may be given for results from Athena Diagnostics NewbornDx.

Electronic Case Report Form Submission Schedule

[X] =Data reported as applicable

Data Case Report Forms	Time point					
	Screening	Enrollment	Return of Result	Transfer and/or Discharge Home	Follow-up #1-11	1 Year CGA
	Forms Required for All Subjects Approached					
Demographics	X					
System(s)	X					
Consent	X					
	Forms Required for All Subjects Enrolled					
Enrollment		X				
Urgent Testing		X				
Source Documents		X	X	[X]	[X]	[X]
Prenatal History		X				
Birth History		X				
HPO		X				
Specimens/Results		X	X			
Results Concordance			X			
Clinical Utility Survey			X			
Clinical Actions			X			
Transfer/Discharge				X		
Child-Visual Analog Scale				X	X[1]	X
SF-12				X	X[1]	X
Hospital Bill				X		
CPT				X		
Resource Utilization Survey					X	X
Completion		[X]	[X]	[X]	[X]	X
	Forms Required for All Subjects Enrolled as applicable					
Clinical Genetic Tests		[X]	[X]	[X]	[X]	[X]
Newborn Screening		[X]	[X]	[X]	[X]	[X]
Medications		[X]	[X]	[X]		
Surgeries		[X]	[X]	[X]		
Death		[X]	[X]	[X]	[X]	[X]
Adverse Event		[X]	[X]	[X]	[X]	[X]
Protocol Deviation		[X]	[X]	[X]	[X]	[X]

[1]The Child-Visual Analog Scale and SF-12 are completed at discharge home, then every 3 months and at 1-year CGA.

Other forms utilized by the enrolling site	Screening	Enrollment	Return of result	Transfer and/or Discharge home	Follow-up #1-11	1-year CGA
Site Screening and Enrollment Log	X					
Demographics Source Document*	X					
Documentation of Informed Consent*		X				
Eligibility Checklist*		X				
Informed Consent Checklist*		X				
Documentation of Informed Consent for the Medical Record*		X				
Blood Specimen Collection Source Document*		X				
HPO Source Document*		X				
Athena Diagnostics Laboratory Requisition		X				
RCIGM Laboratory Requisition (printed from RCIGM portal)		X				
Sema4 Laboratory Requisition (Mount Sinai only)		X				
Documentation of Verbal Result*			X			
Parent/Guardian Contact Information Form				X		
Documentation of Follow-up *					X	X
Study Workflow Checklist *		X	X	X	X	X

*Optional use by site if other source documentation or site-specific forms exists

Data sent from each laboratory; data stratified by infant case ID (RCIGM), accession number (Athena) or sample ID.
Preliminary result [gene(s) and variant(s)] prior to confirmation testing
Date and time of sample receipt
Date and time the preliminary result was determined prior to confirmation testing
If the preliminary result was returned provisionally, the date/time the verbal result was called to the site
If the preliminary result was returned provisionally, the date/time the provisional written report was sent to the site
Whether the preliminary result was determined using proband-only, duo or trio data
Whether or not the confirmation testing confirmed the preliminary result
Infant-only analysis for subjects who were initially analyzed as a duo/trio

2 INTRODUCTION

2.1 STUDY RATIONALE

Congenital abnormalities and genetic disorders are a leading cause of infant mortality in the US[1]. While newborn screening (NBS) has dramatically reduced infant morbidity and mortality for some genetic disorders, these improvements have not had a significant impact in Neonatal Intensive Care Units (NICU) where 10-25% of all NICU admissions are the result of a genetic disorder. Infants with a genetic disorder remain in the hospital approximately 40% longer than those without genetic disorders. Due to the non-specific presentation of many of these genetic disorders, many infants do not receive a definitive diagnosis in a timely fashion, if at all.

Undiagnosed genetic disorders contribute significantly to infant mortality and morbidity. Each year, an estimated 14.4% of neonates are admitted to the NICU for treatment of an acute condition, with average medical costs of $76,164 per patient. Overall mortality rates in the NICU vary from 0.8% to 6.2%, increasing to as high as 57% when there are significant delays in appropriate diagnosis[3-5]. Genetic abnormalities (e.g. structural and metabolic disorders) are a leading cause of death in these infants with over 8,500 known genetic disorders as major contributors[5-15]. While we do not yet know the genetic basis of many disorders in neonates, it has been estimated that more than 1,900 genes appear to be relevant in the neonatal period with symptom onset generally occurring prior to 2 years of age. Until the recent development of rWGS, timely molecular diagnosis of many suspected genetic disorders did not occur. In fact, preliminary studies demonstrate that neonates with a genetic disorder risk going undetected or being misdiagnosed, with 45% of diagnoses made via rWGS not included in the clinician's initial differential diagnosis[16]. The result can be a 5-year gap between disorder onset and diagnosis due to: 1) profound clinical and genetic heterogeneity; 2) limited standard genetic testing currently available (e.g. sequential and single gene sequencing); 3) prolonged length of time required to obtain the results of standard genetic tests; and 4) deferral of testing to post discharge since most payers do not reimburse hospitals for genetic testing while a neonate remains hospitalized[17-22].

When performed early in the clinical course, rWGS triples the diagnostic rate compared to SOC[23, 24]. This provides preliminary evidence that rWGS or NewbornDx could be more cost effective and clinically useful than current standard of care (SOC)[25]. Furthermore, data from the NSIGHT study (NCT02225522) indicates a diagnosis is made only 15% of the time in the neonatal period using SOC testing compared to 44% for rWGS[70]. The total cost for clinical rWGS trio is ~ $19,500,[25,26] suggesting that NewbornDx panel followed by a reflex to rWGS (if necessary) may be a more optimal use of resources[25,28-31]. Here we propose to examine the diagnostic yield of NewbornDx and rWGS, assess the clinical utility of genomic testing, and evaluate each technique's relative cost using data collected from the trial along with simulation modeling. The NIH, the National Academy of Medicine (NAM), and several European agencies have consistently identified scientific and operational barriers to implementation of genomic medicine. Specifically, the "burden to clinicians in obtaining, interpreting, and managing

results", including "integrating effective clinical decision support tools into the EMR" are some of the biggest challenges of scaling genomic medicine[32-35].

NewbornDx offers many of the benefits of rWGS by: 1) detecting some non-exonic regions, sequence variants, chromosomal level abnormalities and high homology regions; 2) covering multiple phenotypes; 3) improving interpretability; 4) lowering costs; 5) obtaining relatively rapid results and 6) expanding utilization worldwide (potentially conducting the panel on every neonate as the next generation of NBS). NewbornDx and rWGS also offer future opportunities to incorporate other features such as the ability to detect variants that alter protein abundance, initiation codons, stop codons and triplet expansion repeats. These future enhancements will assist neonates who currently cannot be confirmed as positive when there is a single heterozygous pathogenic variant in an autosomal recessive disorder and a second variant cannot be detected. However, unlike rWGS, NewbornDx will not include newly discovered disorder genes (although they can be added in the future). rWGS is more comprehensive and covers larger areas of the genome allowing examination of intronic regulatory variants compared to NewbornDx. It is therefore very timely to understand the diagnostic yield of rWGS and NewbornDx, the clinical utility of rapid genomic testing, and the cost-effectiveness of the approaches compared to each other and the current standard of care.

Since disorder progression can be extremely rapid in neonates, a molecular diagnosis must be made as soon as possible in order to impact outcome[19,20,24,26,33,34]. Stark, et.al performed a study supporting the utilization of molecular diagnostic platforms to improve outcomes, reporting a 58% diagnostic rate in neonates who underwent WES compared to a 17% diagnostic rate in neonates who underwent standard genetic investigations[23]. Despite these high-yield diagnostic rates, the average time-to-molecular diagnosis using conventional WGS and WES is prolonged (>3-4 weeks), limiting their clinical utility. In particular, neonates receiving an early molecular diagnosis (37%) did better than those diagnosed later (20%). Initiation of palliative care and avoidance of major morbidity were higher among those with an early diagnosis (17%) compared to those with a later diagnosis (9%). Overall, these studies suggest that outcomes can be significantly improved with earlier diagnosis (similar to NBS). NBS identifies 12,500 affected babies per year at <10 days of life or about 0.3%[35]. Although rapid diagnosis through NBS is known to save thousands of lives per year, it represents just a small fraction of the genetic disorder burden in neonates and is limited to detection of protein levels using Tandem Mass Spectroscopy. In addition, these protein levels are often adversely affected by the treatments delivered in the NICU such as total parenteral nutrition. By enhancing diagnostic yield, cost-effectiveness, and time to diagnosis, NewbornDx or rWGS could facilitate more rapid initiation of appropriate therapeutic interventions or redirection of the goal of care from cure to comfort and/or the withdrawing of life-sustaining treatment[5,24,36-41]. The goal of a precision medicine approach in the NICU is to apply rapid molecular diagnostics in order to supplement empiric, phenotype-driven management with genotype differentiated treatments.

Innovation

- The NewbornDx panel covers 98.4% of the genes in which sequence variants may cause known neonatal onset phenotypes.

- The NewbornDx panel, like rWGS, offers opportunities for future improvements through the addition of newly discovered genes as well as the development of features that allow genome enrichment on the protein level that are complementary to NBS.
- Simple clinical reporting formats have been developed where results can be delivered electronically to the point of care through the EMR and easily referenced for future use.
- Our approach will establish interoperability and scalability across all healthcare institutions even where genetics expertise may be limited. This critical step towards implementation is vital since the vast majority of neonates are admitted to NICUs where there may be limited access to experts in Medical Genetics.
- The incorporation of NewbornDx or rWGS into neonatal care does not have to impact the infant's future with information regarding possible later adult onset diagnoses.
- This prospective, multicenter trial will provide the first prospective comparison of NewbornDx and rWGS in NICUs with long-term follow-up to elucidate risks vs. rewards, costs vs. benefits, and impact on morbidity and mortality.
- Neonates at the six CTSI sites are ethnically and racially diverse, which will address ongoing criticism of existing rWGS trials that lack sufficient ethnic/racial diversity to insure generalizability.

2.2 BACKGROUND

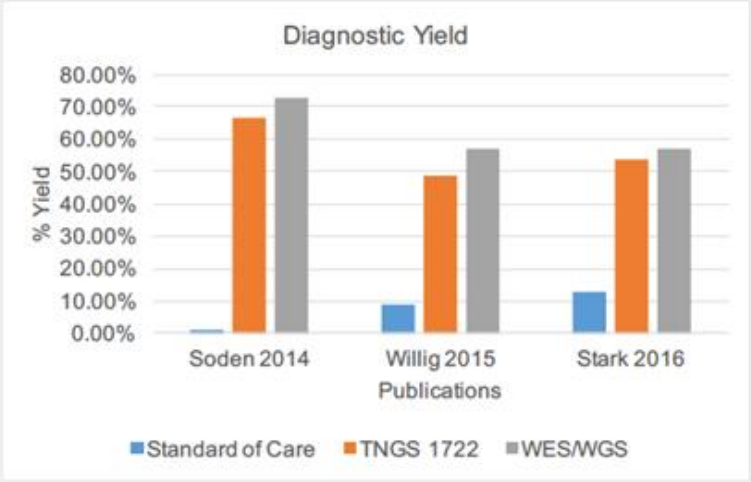


Figure 1: Comparison of diagnostic yield for standard of care (blue), exome/genome sequencing (grey), and in silico TNGS panel (orange) for 254 patients across three publications

In-silico comparison of diagnostics rates demonstrate that a rapid neonatal NewbornDx (aka TNGS) panel may detect up to 90% of the same disorders as WGS at substantially lower cost (**Figure 1**).

We performed a retrospective in silico comparison of the 1,722 gene NewbornDx panel to already published rWGS and rWES clinical data from three studies representing 67 patients and 75 variants[19,20,33]. Compared to WES/WGS, the NewbornDx panel would have detected 85-94% of the same variants. A rapid NewbornDx panel using DBS has already been clinically validated under an SBIR Phase I grant through NICHD[42]. The panel is available as a laboratory developed test and is composed of 1,722 genes representing disorders that affect all systems (**Figure 2**), including all coding regions and clinically relevant non-coding regions.

Figure 2. NewbornDx panel representing all 16 systems

Audiological
Hearing loss (syndromic): 127 genes
Structural abnormalities: 24 genes

Cancer Predisposition
Neuroendocrine/CNS: 22 genes
Hematologic: 50 genes

Metabolic
Mitochondrial: 132 genes
Carbohydrate metabolism disorder: 23 genes
Lysosomal storage disease: 38 genes

Neurological
Brain malformations: 196 genes
Seizures: 304 genes
Microcephaly: 266 genes

Hematologic
Anemia: 82 genes
Bleeding disorder: 29 genes
Platelet disorder: 35 genes

Gastrointestinal
Large and small intestine: 10 genes
Liver disease: 42 genes
Failure to thrive: 18 genes

Skeletal
Short stature: 84 genes
Contractures: 31 genes
Bone fragility: 29 genes

Respiratory
Respiratory distress: 39 genes
Abnormal breathing: 12 genes

Muscle
Muscle atrophy/dystrophy: 44 genes
Hypotonia: 81 genes

Skin
Abnormal pigmentation: 25 genes
Abnormal hair and hair growth: 18 genes
Blistering: 18 genes

Endocrine
Adrenal dysfunction: 15 genes
Hypo-/hyper-glycemia: 44 genes
Hyper-/hypo-parathyroidism: 32 genes

Immune
Recurrent fever/recurrent infections: 18 genes
Immunodeficiency: 56 genes

Cardiac
Cardiomyopathy: 106 genes
Structural defects: 108 genes
Conduction defects: 107 genes

Genitourinary
Renal disease: 115 genes
Genital abnormalities: 37 genes

Ophthalmologic
Abnormal eye movements: 46 genes
Anterior chamber dysgenesis: 27 genes
Optic nerve abnormalities: 42 genes

The phenotypes for most of these disorders typically present before 2 years of age. Specifically, of the 2,300 genes with neonatal onset phenotypes in OMIM, 1,750 are the result of sequence variants (76%) and 150 are the result of copy number variants (CNVs) (6%). There are an additional 150 tumor cell line genes (7%) and 250 genes (11%) with benign phenotypes. Eliminating the latter two groups leaves 1,900 genes with neonatal onset phenotypes, from which we have curated the final list of 1,722 genes while eliminating portions of the genome that are challenging to sequence on any platform. The result is a comprehensive NewbornDx panel that represents 98.4% of the known genes in which sequence variants result in disorders presenting in the neonatal period (**Figure 3**).

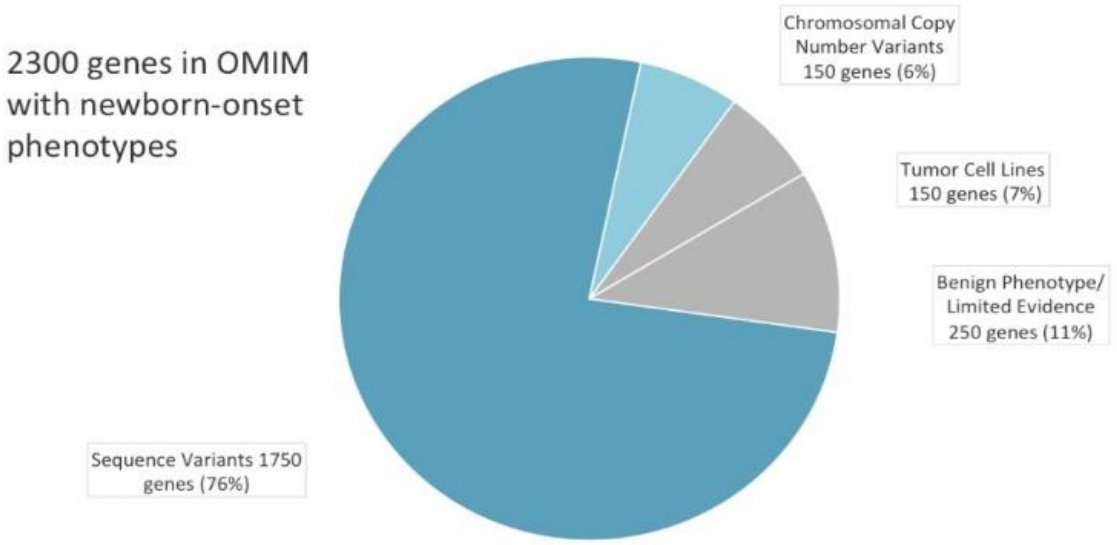


Figure 3: Contribution of variant types to conditions with neonatal-onset presentations.

The ability of NewbornDx to detect CNVs increases this number to 98.5% of total genes known to cause neonatal disorders. The panel shows analytic sensitivity of 98% for single nucleotide variants with an average depth of coverage of 139x (>97% of bases are covered at 20x). Extraction of DNA from DBS has also been validated and can be performed using an existing sample collection kit.

In preliminary studies, NewbornDx compared favorably to rWGS, and SOC. We performed NewbornDx on 10 trios from Pittsburgh's Children's Hospital (our collaborator Dr. Vockley) and 6 patients from Rady Children's Hospital (Dr. Kingsmore). Neonates were chosen based on the clinical suspicion of an underlying genetic condition with symptom onset early in life. Results were returned within an average of 13 days. We found 7 patients with causative variants for a diagnostic yield of 47%, in line with that observed in previous publications using WES and WGS. One of the diagnoses was a condition not included in the pre-test differential diagnosis, consistent with the findings in Petrikin et al.[16]. Results from Dr. Kingsmore's study (NCT02225522) suggest NewbornDx may have detected 100% of diagnostic findings generated using WGS[33].

There is a strong need for prospective studies examining the clinical utility of NewbornDx and rWGS in neonates. While studies have compared WES/WGS in the NICU, they lacked generalizability to a larger population since: 1) they were underpowered or were not prospective, 2) patients were selected based on a positive family history, or 3) the variants were detected in previously characterized genes and therefore potentially detectable on the NewbornDx panel, 4) they did not utilize recently

developed rapid methods for rWES or rWGS[24,25,27,33,43]. As such, no prospective assessment of the clinical utility, cost-effectiveness or outcomes associated with a NewbornDx-based diagnosis of genetic disorders has been conducted. Such evidence is critical to justify the use of the methodology given the cost limitations imposed for hospitalized neonates (inpatient genetic testing is expensive, often bundled, and usually not reimbursed independently). In particular, no study has examined the temporal dynamics of genetic disorder progression in the most vulnerable neonates and defined specific metrics to evaluate how NewbornDx and/or rWGS will best guide treatment and reduce morbidity and mortality. Finally, there are limited studies defining best practices related to NewbornDx or rWGS with regard to communicating information to the medical team and family. This includes: 1) providing timely results with appropriate genetic counseling, 2) reviewing information about available clinical trials and/or approved drugs to treat the condition, or 3) offering redirection of care from cure to comfort and/or withdrawing of life-sustaining treatment. We propose a prospective study of the effectiveness of NewbornDx and rWGS in infants admitted to the hospital with a suspected genetic disorder. This transformative study represents one of the first times key stakeholders with expertise in neonatal clinical trials, medical genetics, and molecular diagnostics are participating in a prospective study of this size and scope.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Blood Sampling: Blood samples should pose minimal health risks to participants. Every attempt will be made to obtain blood from the enrolled infant at the time that routine samples are collected for clinical indications. For the infant, a minimum of two and a maximum of five dried blood spots are needed for NewbornDx, a total blood volume of 1mL for rWGS. This one-time blood sample from the infant should not cause undue trauma or excessive blood loss. If the clinical team does not feel it is safe to obtain the sample from the infant, then we will obtain the blood just prior to a packed red blood cell transfusion. The 3ml of blood from each biological parent is considered minimal for an adult. Risks associated with blood draws are usually minor and include pain, anxiety, bleeding, bruising, fainting, and rarely infection at the site. All samples will be collected by qualified staff following hospital guidelines and standard precautions.

Loss of confidentiality: Since we are storing identifying information about the infant and parents, there is a small risk that this information may be compromised. All study related material will be stored securely. Each enrolled infant will be assigned a unique study identification number which does not include any identifiers. Infant information entered into the password-protected REDCap System and documents used for remote monitoring will be identified by the study ID number. Each site will keep the link between the study ID number and identifiers for all subjects enrolled at their site in a secure password-protected spreadsheet. Blood samples, medical information provided on the laboratory requisition form, sequencing data, and results from the laboratory will be identifiable by name and date of birth. This is to ensure that: 1) the correct infant can be identified immediately 24/7 in the case of a time sensitive result that could influence

survival and outcome; and 2) to eliminate any errors with matching of study ID to infant at the enrolling site. The ICF clearly indicates that the labeling of specimens, medical information, and reports to/from the laboratories will contain identifying information. All genomic sequencing data will be electronically stored at the laboratory that conducted the test in password-protected databases accessible only to research personnel. In addition, this study is automatically issued a COC. PHI related to a genetic diagnosis may need to be shared with government agencies or other organizations involved in the research. WGS may uniquely identify any person. Since a COC may not apply in all circumstances and WGS can identify any person, it is essential that this be included in the ICF.

Secondary findings for the infant and biological parent(s): NewbornDx and rWGS may detect secondary findings. These include diagnoses for the infant or a biological parent not related to the infant's symptoms, misattributed parentage, and suspected incest. Parents/guardians of infants will be counseled prior to enrollment about the information available from each platform.

The infant's parent/guardian will choose whether or not to receive secondary diagnoses for the infant. This will be by an opt-in or opt-out (express choice of one or the other) included in the ICF. A secondary diagnosis for the infant will only be reported if both criteria 1 and 2 are met and at least one of 3a or 3b:

1. The parent/guardian opted to receive secondary findings for the infant, AND
2. The disorder onset may occur in childhood, AND
3. At least one of the following:
    a) The disorder is medically actionable AND/OR
    b) The disorder involves a gene on the "American College of Medical Genetics and Genomics (ACMG) list" of genes recommended for reporting of secondary findings at the time of testing[46]

It is possible a secondary diagnosis will eventually become a primary diagnosis (in the case where the infant has not yet grown into the phenotype, an atypical presentation, or the field advances) and the parent/guardian will not have been given the relevant information because the parent/guardian elected not to receive secondary findings for the infant. Information about conditions with an onset occurring only in adulthood will not be given for the infant.

If the parent/guardian opted-in for infant secondary findings, a biological parent will have the choice to opt-in on the ICF to receive secondary findings. A secondary diagnosis for a biological parent will only be reported if the biological parent opted in to receive secondary findings and the disorder involves a gene on the ACMG list recommended to report as secondary findings at the time of testing. There are some circumstances in which a biological parent of an infant could learn about a current condition or an increased risk for an adult-onset disorder even if they do not opt-in to receive secondary findings by the inheritance and penetrance pattern of the infant's disorder.

Misattributed parentage (mistaken paternity or an egg donor) will not be reported by the laboratory or the enrolling site. The ICF indicates that misattributed parentage could be inferred from the results. For example, the infant is found to have an autosomal recessive condition and one parent is found not to carry this condition and de novo mutations are known to be rare. Additionally, the ICF states that there are privacy risks if the parent/guardian and/or biological parent receives the raw sequencing data and gives it to another person for analysis. Privacy risks which include misattributed parentage will be explained if a parent/guardian requests to receive raw sequencing data.

This testing could detect incest, defined as "a sexual relationship between people too closely related to marry each other (such as a person having a sexual relationship with a parent, grandparent, brother, or sister)." If incest is suspected, this information will be reported to the site. The site will notify child protective services. Risks of discovering and reporting incest may include possible criminal charges depending upon state law. Risks to the infant include behavioral problems and removal from the home. The capability of the sequencing tests to detect incest, the legal requirement to report it to child protective services and associated risks is in the informed consent form.

The ICF states that secondary findings in the infant or biological parents is not the primary purpose of this research study and just because a secondary finding isn't reported doesn't mean any disease-causing genetic changes don't exist.

Misinterpretation of results: We expect to miss a small number of structural variants (SVs) using short read rWGS (NewbornDx does not detect structural variants) that are causal. We will monitor the percentage of identified SVs and be careful not to label cases with a negative result as not genetic in origin. Experienced personnel will perform the genomic testing according to well-established protocols. Although we may not find a result, this does not mean the infant will not continue to have symptoms or even have a genetic disorder. Similarly, the infant's health may not improve during this study; it may get worse or stay the same. All infants will continue to receive routine care as directed by the clinical team, including routine NBS and other genetic testing.

Risk of discrimination associated with genetic testing: Federal and State laws generally make it illegal for health insurance companies, group health plans, and most employers to discriminate against a person based on their genetic information. The Genetic Information Non-discrimination Act (GINA) indicates that genetic information cannot be used by certain health insurance companies when making decisions about eligibility for health insurance or how much insurance costs. These insurance companies can still use any genetic information to help them decide whether or not they will pay health insurance claims. This law also indicates that employers with fifteen or more employees may not use genetic information when making decisions to hire, promote, or fire an individual or when setting the terms of employment. Protections by this law may not apply to certain military, veterans and federal employees. These laws do not protect against genetic discrimination by companies that sell life, disability, or long-term care

insurance. Information regarding the protections and limitations of Genetic Information Nondiscrimination Act (GINA) will be included in the ICF.

Emotional distress: Genetic testing may cause anxiety or emotional distress due to feelings of responsibility for passing genetic variants to children. If parents/guardians and/or biological parents opt-in to receive secondary findings for their infant and/or themselves, this information may improve their overall health, but could provoke anxiety by knowing the information. Genetic counselors will be available to all parent/guardian(s) and/or biological parents at all times while enrolled in the study to answer questions or addressing concerns related to testing or results.

2.3.2 KNOWN POTENTIAL BENEFITS

This study may or may not benefit the infant. An infant may receive a diagnosis or additional information regarding his or her medical condition. The main benefits to the infant include a faster time to diagnosis, a more accurate diagnosis, and initiation of appropriate interventions faster than possible with standard diagnostic testing. These benefits have the potential to significantly reduce morbidity, mortality, other diagnostic testing, overall hospital costs, and time to initiate appropriate treatment and time to discharge. There will be no direct benefit to participation for unaffected family members except where a diagnosis provides information regarding reproductive decisions and recurrence risks. The targeted nature of the NewbornDx testing enables reporting of results without providing secondary findings that might burden families.

The NewbornDx panel and rWGS may each have specific benefits including:

- NewbornDx only detects variants in genes known to be actionable in the neonatal period.
- NewbornDx avoids the financial burden of rWGS.
- NewbornDx decreases the ethical considerations of rWGS related to secondary findings or findings related to parents; however, these findings can be associated with improved health for patients and families.
- Rapid return of results (either by rWGS and/or NewbornDx)
- rWGS provides coverage of CNVs and other genetic disorders

Importance of the knowledge to be gained:
rWGS is slowly becoming integrated into neonatal care in an attempt to broaden diagnostic coverage while reducing time to diagnosis. However, each platform is expensive, may identify disorders with limited therapeutic options, and uncover adult onset disorders. NewbornDx adopts the philosophy of NBS by providing assessment of 1,722 genes matched to phenotypes presenting in the neonatal period, including those where a timelier intervention can improve outcome. Our preliminary data indicate that NewbornDx may be superior to traditional diagnostic testing and genome scale sequencing by transforming empiric, phenotype-driven management into genotype-informed treatment plans that improve outcome without the ethical, financial and technical burdens associated with WGS. If NewbornDx has a comparable diagnostic yield as rWGS, with favorable cost effectiveness compared with rWGS and standard of care, it could easily be adopted worldwide. This study has the potential to advance the

expanding field of molecular diagnosis in high-risk neonates and even enhance traditional NBS.

2.3.3 ASSESSMENT OF POTENTIAL RISKS

- Drawing of blood can be associated with pain, bruising, anemia, and possible infection.
- A genetic diagnosis could be missed, with clinicians thinking it is unlikely to be present since the genetic testing is negative.
- If parents/guardians and/or biological parents opt-in to receive secondary findings for their child and/or themselves, this information may improve their overall health, but also may provoke anxiety in learning about the information.
- If parents/guardians and/or biological parents opt-out of receiving secondary findings, they will not receive information that could potentially impact their health or their child's health.
- Despite the presence of a COC, PHI related to a genetic diagnosis may need to be revealed to government agencies or other organizations involved in the research.
- For a critically ill infant, a preliminary diagnosis may be disclosed to the clinical team without time for confirmatory testing. Potential therapy may be initiated that may not be necessary.
- If a diagnosis is made, the information may not be provided to the clinical team in sufficient time to improve outcome.
- It is not clear how reporting of variants of unknown significance will impact care and outcome.

3 OBJECTIVES AND ENDPOINTS

Primary Objectives	Endpoints		Endpoint Justification
To estimate the diagnostic yield of NewbornDx and rWGS in identifying genetic disorders of unknown etiology	Primary	A confirmed genetic disorder detected by NewbornDx A confirmed genetic disorder detected by rWGS	Assess diagnostic capability of the platforms
	Secondary	For diagnoses by rWGS, if trio testing was required to confirm a diagnosis	Assess need for including biological parents
To assess the clinical utility of genomic sequencing	Primary	Time from sample collection to positive test result	Assess the timeliness of an accurate result
	Primary	Clinical utility of genomic sequencing	Assess impact on care
	Secondary	If the infant does not receive a diagnosis, calculated at discharge and at 1year CGA	Assess the clinical impact of platform(s)
Secondary Objectives	Endpoints		Endpoint Justification
To examine the economic impact and health outcomes of NewbornDx and rWGS in infants compared with SOC diagnostic testing, over a one-year and lifetime horizon	Secondary	One-year cost-effectiveness of NewbornDx and rWGS compared with SOC testing	Assess economic value of alternative testing strategies
	Secondary	Lifetime cost-effectiveness of NewbornDx and rWGS, compared with SOC testing	Assess economic value of alternative testing strategies
To develop and qualitatively assess the web-based clinical reference database of supporting information for medical providers and parents	Secondary	Satisfaction of the web-based clinical reference database	Assess ease of use and usefulness of information among medical providers
Exploratory Objectives	Endpoints		Endpoint Justification
To inform the NewbornDx panel with findings from rWGS that were not identified with NewbornDx	Exploratory	Conditions detected by rWGS that were not detected by NewbornDx	Expand and improve the diagnostic accuracy of the NewbornDx panel

How endpoint(s) will be adjudicated

1. Confirmed genetic disorders detected by NewbornDx or rWGS: the laboratories conducting testing could report descrepant results due to differences in confirmatory methodology, variant types or methods of interpretation. Methods used for confirmation testing at each laboratory will be documented. If results are discordant due to a variant classification, the laboratories will each provide the evidence supporting their result and variant classification. This summary will be given to the enrolling site PI to share with the clinical team; a subsequent phone call with the labs may be arranged upon request from the site. The clinical team who is caring for the infant is in the best position to determine if one result aligns best with the infant's clinical presentation and their view will be captured in the study data. Additionally, a subset of the study geneticists on the steering committee who are independent of the labs will review the redacted lab reports and lab summaries and provide what they think is the most accurate result for an additional study endpoint. The labs are available for questions by the steering committee. The opinion of the steering committee members may be shared with the enrolling site upon the site's request. Detailed procedures are outlined in the MOP.
2. Clinical utility of genomic sequencing testing: it is possible that the clinician completing the clinical utility survey (refer to section 8.1) might indicate an opinion or action that differs from the medical record data abstraction reported by the study coordinator on the Clinical Actions after Genomic Sequencing form. In such a case, the research team at the site will review the relevant information with the clinical team to find out and document the reason for the discrepancy and if applicable, make any corrections (i.e. a survey completed for the wrong infant).

4 STUDY DESIGN

4.1 OVERALL DESIGN

This multicenter, prospective, clinical trial will estimate the diagnostic yield of NewbornDx and rWGS testing and assess the clinical utility of genomic sequencing among high-risk infants with signs/symptoms consistent with a possible genetic disorder. The relative cost effectiveness of each testing method will be evaluated through simulation.
There are three components to this study:

1. Infants will undergo SOC diagnostic testing as well as NewbornDx and rWGS in parallel. Genetic sequences via NewbornDx and rWGS will be generated for the infant (proband) and when available, the biological parents, and analyzed together. NewbornDx and rWGS test results will be returned to the infant's clinician, medical record and parent/guardian. Those infants analyzed as a duo or trio will have a retrospective analysis done of only the infant's data to determine if the same result would have been obtained with and without parent samples. Clinical utility will be measured by clinician opinion and changes in the infant's care as a result of the genetic sequencing test results. QoL and medical resource utilization will be collected by parent survey monthly, until the infant is one year CGA.
2. A retrospective chart review of neonates with suspected genetic disorders completed at 3 sites, will be used to elucidate 1-year cost and health outcomes that would have been incurred in the absence of available NewbornDx and rWGS testing. The retrospective review will include 300 neonates born 12-18 months prior to the start of the study and performed under a separately submitted amendment to the central IRB. The cost and health outcome data will be used in an economic evaluation comparing NewbornDx, rWGS, and SOC over 1-year and a child's lifetime time trajectory.
3. A user-friendly clinical reference database will be developed to provide medical providers and families with information about genetic disorders, treatments, clinical trials and advocacy groups. A qualitative assessment of the clinical reference database will be conducted through surveys of clinicians involved in the infants' care. Once the database is completed and prior to implementation of the clinical reference database into this study, an amendment will be submitted to the IRB along with the survey.

Hypothesis #1: NewbornDx will detect at least 60% of the diagnoses identified by rWGS

Hypothesis #2: NewbornDx will be substantially less costly than rWGS or SOC testing over a one year and lifetime horizon

Hypothesis #3: Medical providers will be satisfied with the web-based clinical reference database of supporting information.

Interim analyses are not planned.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

There is a strong need for prospective, comparative studies comparing the applicability of NewbornDx and rWGS in the neonatal population. While studies have been done comparing WES/WGS in the NICU, they lacked generalizability to a larger population since: 1) they were underpowered or were not prospective, 2) patients were selected based on a positive family history, or 3) the variants were detected in previously characterized genes and therefore potentially detectable on the NewbornDx panel. As such, no prospective assessment of the clinical utility, cost- effectiveness, or outcomes associated with a NewbornDx-based diagnosis of genetic diseases has been conducted. The proposed study design will maintain equipoise with direct comparative analysis between two comprehensive genetic sequencing approaches for use in infants in an attempt to determine their cost-effectiveness and diagnostic yield for use in this vulnerable population.

4.3 END OF STUDY DEFINITION

An infant is considered to have completed the study if he or she has undergone NewbornDx and rWGS and completed the last interview at 1 year CGA or died prior to1 year CGA.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an infant must meet all of the following criteria:

1. Documented informed consent from the parent/guardian
2. Signs/symptoms consistent with a possible genetic disorder including, but not limited to dysmorphic features, congenital anomalies, hypotonia, seizures, clotting, poor oral feeding skills
3. Admitted to a hospital (NICU, PICU, CVICU, inpatient floor) participating in this study at the time of enrollment (enrollment is not limited to the initial hospitalization after birth, it may occur during a readmission)
4. Less than one year CGA

There is not a requirement for clinical genetic testing prior to enrollment. However, enrollment in this study is not intended to replace targeted available genetic testing for specific suspected disorders that match the phenotype of the infant and make rWGS testing unnecessary. Examples include trisomies or a high likelihood of DiGeorge syndrome. In these cases, targeted genetic testing for a suspected disorder should be done prior to enrollment (e.g. DiGeorge syndrome and FISH for 22q11.2 deletion). Clinical genetic testing does not need to be completed prior to enrollment if there is more than one diagnosis on the differential diagnosis list. Other than these few situations, our goal should be to understand the role of utilizing rWGS and NewbornDx as a first line choice of testing.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. A known genetic diagnosis (e.g. prenatal testing, biochemical testing and/or enzyme analysis)
2. Major congenital anomaly (renal, cardiac, hepatic, neurologic, or pulmonary malformations) associated with a chromosomal anomaly detected on prenatal testing (e.g. ultrasound, genetic testing)
3. Presence of documented congenital infection (e.g. congenital cytomegalovirus)
4. Infants considered non-viable due to prematurity (< 23 0/7 weeks GA)
5. Infants who are not expected to receive medical care in the US healthcare system from time of discharge home until 1 year CGA

Lack of participation by one or both biological parents in the trio analysis does not preclude the infant from participating in this study. This situation could occur if a biological parent does not provide consent for his/her own blood sample to be used for a trio analysis, if a blood sample cannot be obtained, or in the case of an egg or sperm donor.

5.3 SCREEN FAILURES

Screen failures are defined as infants whose parent(s) consent to participation in the clinical trial but who do not have NewbornDx or rWGS performed. This could occur if a clinical diagnosis was determined after consent was obtained but prior to the start of any study procedures (section 7 addresses the procedure for subject withdrawal if a clinical diagnosis is determined after study procedures commence but before the completion of genomic sequencing). A minimal set of screen failure information will be collected including demography, screen failure details and eligibility criteria.

5.4 STRATEGIES FOR RECRUITMENT AND RETENTION

The anticipated number of infants to be screened is approximately 1200 in order for 400 infants to complete the study. Six US sites will recruit for this study. The parent/guardian(s) of all eligible infants will be approached. Enrollment is expected to include about 50% of each sex and proportions of each race that are represented in the general population in the multiple sites where the studies will be performed. The entire ICF will be translated into Spanish and short forms in other non-English languages will be utilized.

This study involves monthly follow-up from discharge home until 1 year CGA. In order to reduce the risk of lost to follow-up, study staff will have the parent/guardian(s) fill out the Follow-up Contact Information form at the time of transfer or discharge home. This form will document several methods of contact for the parent/guardian(s) and a family member or friend who would know how to reach the parent/guardian(s) if their contact information changes or they are unreachable. Then, the study coordinators will contact the family monthly until 1 year CGA to complete the parent surveys/interviews.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

NewbornDx and rWGS are CLIA/CAP certified and will be completed for all infants. The Athena (Quest) and RCIGM laboratories will be blinded to each other's result until all final results are returned.

Phenotype Extraction and Provision of Clinical Information to the Laboratories

1. A sub-group of clinicians from each site will manually review the medical record, extract relevant clinical information and determine the Human Phenotype Ontology (HPO) terms (refer to MOP). The sub-group of clinicians will consist of a minimum of 3 clinicians with at least one a geneticist or genetic counselor.
2. A small portion of the medical records (genetics consult note, other sub-specialty note (neurology, hematology, etc.) and NICU notes will be selected.
3. The HPO terms and medical records will be provided by the sites to the laboratories via upload to the RCIGM ordering portal. Athena will receive this data to its systems through a secure FTP site or encrypted email. Athena will not have access to the rWGS results through this mechanism.
4. Once HPO terms are submitted by the site, the laboratories may either add additional terms based on the last consult/progress notes submitted by the site or contact the site research team for clarification of symptoms. If this happens by either Athena (Quest) or RCIGM, the additional or revised HPO terms needs to be redirected to the site for upload to the portal. This ensures any new information is shared with both laboratories.

Athena Diagnostics (Quest)

NewbornDx Methods and Workflow

Rapid NewbornDx will be performed at Athena (Quest) Diagnostics to CLIA/CAP requirements. DBS will be collected on filter paper and sequenced. Custom oligonucleotide probe libraries (Agilent SureSelectQXT) will be utilized to capture genomic DNA regions and targeted libraries created as described earlier[42]. The content of the assay (regions of interest) will remain constant for participants in the study. Sequencing of the targeted libraries will be performed on a NextSeq 500 (Illumina, San Diego, CA). Sequencing reads will be aligned to the reference genome (hg19/GRCH build 37) using Burrows-Wheeler Aligner for short alignments (BWA-MEM) followed by a Genome Analysis Toolkit v3.6 (GATK) haplotype variant caller running on the Edico Dragen™ Platform, and annotated with Enterprise genome interpretation software (Fabric Genomics, Oakland, CA). All subject FASTQ, Variant Call File (VCF) and other data files will be securely stored within the Athena (Quest) Diagnostics Isilon storage.

Athena (Quest) Genomic Data Analysis
Data analysis will be completed by a custom bioinformatics pipeline utilizing Burrows-Wheeler Aligner & Genome Analysis Toolkit v3.6. All reported DNA variants will be reviewed by at least one trained genomic variant scientist, one genomic science specialist, and one board-certified laboratory director prior to the final report being issued.

Athena (Quest) Variant Pathogenicity Determination
Variant pathogenicity is assessed following a standardized evaluation protocol[71] consistent with the ACMG standards and guidelines for the interpretation of sequence variants[49]. Data is gathered from multiple sources, including but not limited to, internal datasets, published literature, public databases, and in-silico prediction tools. Evidence is reviewed and assessed by a genomic variant scientist using a standard operation procedure. The proposed pathogenicity classifications derived from the data are reviewed and approved by a board-certified laboratory director.

Athena (Quest) Variant Causality Determination
Variant causality will be determined by the infant's clinical presentation, family history, and any other laboratory results provided. Relevant HPO terms (see phenotype extraction above) will be used with Phenomizer or Phenolyzer to create a differential diagnosis based on the neonate's clinical presentation. Phevor (Fabric Genomics) will be used to prioritize DNA variants based on the genes' associated phenotypes compared to the HPO terms provided by the site. Fabric Genomics will also be used to filter variants based on variables such as inheritance pattern, frequency of the DNA variant in the general population, variant type (missense, nonsense, etc.), and presence in a variant database (i.e. ClinVar or HGMD). Variant filtering will be based on a standardized set of criteria used by genomic variant scientists and board-certified laboratory directors. Sample reports will be drafted and reviewed by a team composed of genomic variant scientists, genetic counselors, and a board-certified director prior to being released to the client.

RCIGM-CGC
rWGS Methods and Workflow
rWGS will be performed at the CLIA-certified, CAP accredited Rady Children's Institute for Genomic Medicine – Clinical Genome Center (RCIGM-CGC). Sequencing is performed in accordance with the RCIGM- CGC standard operating procedures and validations. Briefly, genomic DNA is extracted from whole blood, PCR-free DNA libraries are made, and quality checks performed for library size and concentration. DNA libraries are then loaded on a NovaSeq 6000, and 2 x 100 nt sequencing to an average genome wide coverage of 40x. Short Tandem Repeat (STR) genotypes will be used to assure sample identity is maintained throughout the laboratory process. For each infant or biological parent sample, STR genotypes are generated by a commercial PCR amplification kit and are compared with those called by WGS process. Bcl files are automatically aligned to hg19/GRCH build 37 and nucleotide variants and small insertions and deletions are called with DRAGEN (Edico Genome) in ~1 hour. Files are uploaded to DNAnexus, where structural variants (SVs) are called using the union of

two algorithms (CNVnator and Manta). Redundant SVs are removed from the superset of SV calls. SVs will be annotated to determine affected genes and regulatory regions and compared to public and internal databases to determine MAF and filter common, or false positive SVs. A combined VCF (SNVs/indels and SVs) are uploaded to a rapid instance of Opal (Fabric Genomics), allowing annotation and display within 1 hour.

rWGS Trio Testing
When samples for the infant and parent(s) are received at the same time, RCIGM will generate infant and parent sequences simultaneously and the data from the infant and parent(s) will be analyzed together as a duo/trio. When infant samples are sent without the parent samples, RCIGM will sequence the infant sample and analyze the infant's data. If a diagnosis is not determined or a VUS is identified after analyzing the infant alone, the parents' samples, if subsequently become available, will be sequenced and data from the duo or trio analyzed together. The infant's report of results will indicate whether or not trio testing was done. For all positive diagnoses made when only sequencing and analyzing the infant, the lab will subsequently run testing on the parent samples (if available) to determine inheritance (for example, targeted Sanger sequencing).

RCIGM-CGC Genomic Data Analysis
Data analysis will follow the standards set by the clinical diagnostic laboratory and in accordance with recommendations from the American College of Medical Genetics and Genomics (ACMG/AMP). A minimum of two expert data analysts will be responsible for analyzing the DNA variants following genomic sequencing. Analysts will be rotated among qualified staff, including but not limited to laboratory directors, genetic counseling staff, and trained medical and research staff. One of the analysts will then compile a report to be reviewed and reported by an ABMGG board-certified laboratory director.

RCIGM-CGC Variant Pathogenicity Determination
The laboratory directors as well as Medical Geneticists and Genetic Counselors at RCIGM and at each site will assess pathogenicity of variants according to ACMG/AMP recommendations for classifying sequence. New statistical approaches for variant classification are currently being actively developed by several groups and may be introduced upon verification of their utility. Knowledge of the pathogenicity of individual SNVs and SVs is rapidly evolving through community curation via HGMD, ClinVar and DECIPHER50 and through exhaustive mutant generation and functional testing for individual disorder genes. Variant classification will be performed using expert analysis of all data regarding each variant, including review of available literature and functional models.

RCIGM-CGC Variant Causality Determination:
Variant causality will be determined on a case by case basis depending on the infant's clinical presentation, family history, and any other laboratory results provided. Relevant HPO terms will be used with software tools, such as Phenomizer or Phenolyzer or standard gene lists for common presentations (e.g. neonatal seizures, cholestasis, and

heterotaxy), to create a differential diagnosis list based on the neonate's clinical presentation. Inputs for causality determination will be the annotated genomic variant file and the comprehensive differential diagnosis gene list. Alternatively, the Phevor tool in Opal[52, 53] will be used to rank genes with relationship to the observed phenotype based on their known or suspected association(s). By allowing dynamic filtering of variants based on variables such as individual clinical features, disorders, genes, genotype, and inheritance pattern, Opal will assist in identification of a molecular differential diagnosis. Custom Opal settings can be saved, which allows configuration in a manner that can enable a provisional diagnosis to be determined rapidly. Opal also allows data mark-up, user tracking and data basing of variants and their classifications and export of fields in formats suitable for inclusion in diagnostic reports. In a typical interpretation session, variants will be filtered by allele frequency and presence in variant databases and prioritized based on phase, parent-of-origin, clinical features, and other characteristics unique to the infant or to the biological parents. All potential genetic inheritance patterns will be examined, including de novo, autosomal recessive, autosomal dominant, X-linked, mitochondrial, and, where possible, somatic variation (where trios may be needed). Where a single likely causative heterozygous variant for a recessive disorder is identified, the entire coding domain will be manually inspected using the Integrated Genome Viewer (IGV) for coverage and to screen for additional variants that may not have been called. Expert interpretation will be performed for all likely causative variants with regard to evidence for pathogenicity and causality. Likewise, the causality of structural variants in rWGS is assessed based on frequency in the RCIGM-CGC database and location with respect to the comprehensive differential diagnosis gene list[54]. This is an evolving area of interpretation. SV and nucleotide variant causality is examined together in order to identify compound heterozygosity in recessive disorders.

Secondary/Incidental Findings for the Infant

Secondary/incidental findings for an infant will not be actively sought. A dedicated analysis of the ACMG 59 secondary gene list will not be done. If a secondary/incidental finding is found during the infant analysis, the laboratory will only report it if both criteria 1 and 2 are met and either 3a or 3b:

1. The parent/guardian opted to receive secondary findings for the infant, AND
2. The disorder onset may occur in childhood, AND
3. At least one of the following:
   a) The disorder is medically actionable AND/OR
   b) The disorder involves a gene on the "American College of Medical Genetics and Genomics (ACMG) list" of genes recommended for reporting of secondary findings at the time of testing

Secondary findings of conditions with an onset only in adulthood will not be returned for the infant. Only pathogenic or likely pathogenic variants are reported as a secondary/ incidental finding if they have the potential to impact the infant during childhood.

The "ACMG list" refers to the current list of genes recommended to report as secondary findings. WGS covers all 59 genes currently recommended by the ACMG to return as

secondary findings, NewbornDx covers 30 of those genes. There are 52 genes on the ACMG list that may occur in childhood. In order to account for how the ACMG list might evolve during the study period, the ACMG list at the time testing is done will be used. Therefore, the list used for the first infant enrolled may be different than the list used for the last infant enrolled. If the ACMG list evolves during the study period, we will not retrospectively change what was initially reported; this aligns with current clinical practice.

Primary and secondary findings for the infant, if reportable, will be included in one report from each laboratory.

Secondary Findings for a Biological Parent

A secondary finding for a biological parent may be identified through WGS when conducting the infant's phenotypically driven analysis. Secondary findings for a parent will not be actively sought and a dedicated analysis of the ACMG 59 secondary gene list will not be done. There may be cases in which a biological parent elects to receive secondary findings and the parent's sample does not undergo WGS. This could occur if the infant's samples have been processed prior to the receipt of the parent samples. Secondary findings for a biological parent will not be reported by Athena Diagnostics.

The laboratory will only report a secondary diagnosis for a biological parent if all of the following criteria are met:

1. The biological parent opted in to receive secondary findings for him or herself. A parent may opt-in to receive secondary findings only if the parent/guardian opted-in for infant secondary findings.
2. The disorder is of a gene on the ACMG list of genes recommended to report as secondary findings at the time of testing.

A separate report must be issued for any secondary findings for a biological parent.

When Incidental/Secondary Findings Are Reported

Secondary/Incidental finding found in Infant and Parent	Did Infant Opt-In?	Report for Infant?	Did Parent Opt-in?	Report for Parent?
Included in ACMG59, adult onset	Yes	No	Yes	Yes, RCIGM*
Included in ACMG59, adult onset	Yes	No	No	No
Included in ACMG59, adult onset	No	No	No	No
Included in ACMG59, childhood onset	Yes	Yes	Yes	Yes, RCIGM*
Included in ACMG59, childhood onset	Yes	Yes	No	No
Included in ACMG59, childhood onset	No	No	No	No
Not on ACMG59, childhood onset, medically actionable	Yes	Yes	Yes	No
Not on ACMG59, childhood onset, medically actionable	Yes	Yes	No	No
Not on ACMG59, childhood onset, medically actionable	No	No	No	No

*Athena/Quest will not report secondary/incidental findings for parents.

Confirmatory Testing

With the exception of a provisional result, pathogenic and likely pathogenic variants (as determined by ACMG variant classification criteria) that relate to the neonate's current phenotype as well as suspicious VUS will be clinically confirmed at RCIGM or Athena prior to the return of written results. Additional confirmation testing may be done by Sema4 for subjects enrolled at Mount Sinai (refer to Sema4 section below). Segregation analysis on biological parents will be performed when warranted. A provisional result will be given in cases in which delaying the return of results until confirmatory testing occurs places the infant at an increased risk of morbidity or mortality (a treatment modality is available whose delay in administration could cause irreversible harm). Prior to confirmation testing, the laboratory will return provisional results and management guidance via verbal report with read back followed by a written provisional result report to the enrolling site. Refer to the section, Sema4 and Subjects Enrolled at Mount Sinai, for when provisional results may be given for subjects from Mount Sinai.

Confirmatory testing will subsequently be performed for all provisional results and a written report of the final result issued. The laboratory will make the decision about whether a finding meets the criteria to be given provisionally; if there is a question, the lab will consult with the study-wide PIs. A lethal condition will not be released as a provisional result; it will be confirmed prior to this result reported.

Variant Classification	Is confirmation testing needed?
Pathogenic	Yes
Likely Pathogenic	Yes
Variant of Unknown Significance (VUS)	Only if suspicious (where the phenotypic fit is very good and/or functional confirmatory tests are readily available and/or the results are actionable)
Likely Benign	No
Benign	No

Secondary diagnoses	Is confirmation testing needed?
A secondary or incidental finding that will be reported	Yes

Category	Definition	Detected By NewbornDx	Detected by rWGS	Method(s)
SNP	variation in a single nucleotide	Yes	Yes	Confirmation testing methods are at the discretion of the laboratory director. Common methods include MLPA, microarray, Sanger and qPCR.
Indel	an insertion or deletion of less than 1 kb in length, in-frame and frame shift	Yes	Yes	
Structural Variant	Inversions, balanced translocations, CNVs; ~ 1 kb and larger	No	Yes	

Sema4 and Subjects Enrolled at Mount Sinai

Results returned to the medical record for subjects from Mount Sinai must be from a New York State CLIA certified lab and of a New York State conditionally approved test. Athena Diagnostics is a New York State CLIA certified lab and NewbornDx is a New York State conditionally approved test. Therefore, NewbornDx results for subjects from Mount Sinai will be returned to the research team, clinician(s) caring for the subject, the subject's parent/guardian(s) and placed in the subjects' medical record. Provisional results from NewbornDx may be acted upon prior to Athena's own confirmation testing.

Until the time RCIGM becomes a New York State CLIA certified laboratory and rWGS a New York State conditionally approved or approved test, subjects from Mount Sinai may require additional confirmation testing by Sema4. Sema4 is a New York State CLIA certified and Mount Sinai wholly owned laboratory. Procedures to be followed:

1. The research team at Mount Sinai will send samples to Sema4. Sema4 will extract DNA from the infant and biological parent blood samples and send two aliquots to RCIGM-CGC while retaining an aliquot for possible future confirmation testing. DNA requirements: > 3 ug DNA (concentration ranges from 20 ng/ul to 200 ng/ul) with Picogreen measurement. If the site used non-Picogreen measurement, 4-5 ug DNA (concentration ranges from 20-200 ng/ul).
2. RCIGM will issue a research grade report of its results to the research team at Mount Sinai. This report will have the heading "research report" and also include disclaimers in the Regulatory Disclosures section. The research report will only be placed in the research file and not in the medical record.
3. A provisional result will be given to the research team in order for them to alert Sema4 to start confirmation testing if required (i.e. RCIGM reports a provisional result which is not also reported by Athena). A RCIGM provisional result will not be shared with the clinical team, parent/guardian nor put in the medical record.
4. Pathogenic, likely pathogenic or VUS results returned only by RCIGM will have additional confirmation testing and interpretation done at the Sema4 laboratory. If RCIGM and Athena report the same result, Sema4 confirmation testing is not required. At Sema4, confirmation testing will be done by targeted analysis with Sanger, or for CNVs with qPCR, MLPA, or ultra-high resolution exon array.
5. RCIGM will send final results as a research report to the Mount Sinai research team. The Mount Sinai research team will compare the RCIGM research report with Athena's report and alert Sema4 of confirmation testing as necessary.

RCIGM Result	Agreement with NewbornDx	Is testing by Sema4 required?	What reports are put in the medical record?
Pathogenic, likely pathogenic, VUS or secondary/ incidental result	The findings reported by RCIGM are also reported and interpreted within the similar variant classification categories (P/LP v. VUS) by Quest/Athena.	No	NewbornDx
	RCIGM reports findings that Quest/ Athena did not report.	Yes	NewbornDx, Sema4
Negative Result	Yes	No	NewbornDx
	No	No	NewbornDx

If during the course of this study, the RCIGM rWGS test becomes CLIA certified in New York State and the rWGS test is New York State approved or conditionally approved, the processes involving Sema4 will be eliminated.

Discordant Results
It is possible that the final results of NewbornDx and rWGS may be discordant. It is also possible that the confirmatory testing done by Sema4 for infants enrolled at Mount Sinai will be discordant to the confirmed NewbornDx or rWGS results. This could be a result of both technical and interpretation differences:

- technical differences of the platforms where a specific variant may be identified on one platform but not another
- different genotyping and variant filter thresholds when both platforms return the same variant
- different classification of the same variant as benign, likely benign, VUS, likely pathogenic or pathogenic

Which methods are used for confirmation testing at each laboratory will be documented.

It will be determined if the discordance is caused by the technical performance of the platform or variant filtering or classification. In cases of discordance due to variant classification, each lab will use an agreed upon format to provide a written document summarizing the evidence supporting their variant classification. Refer to section 8.1 and the MOP for adjudication procedures.

Inconclusive Results
Under certain circumstances, a test result may not be conclusive. These situations will be reviewed on an individual basis and testing may be repeated or another sample requested. The reasons for inconclusive testing are explained in the result report.

Requests for Re-analysis of Sequencing Data
Requests for reanalysis can only be made after a significant change in phenotype occurs in the infant (e.g. new onset seizures) when results have already been returned. This may be permitted on a case-by-case basis only after discussion between the enrolling site PI and study-wide PIs.

Retrospective Singleton (Proband) Analyses for Duo/Trios
After the final result is returned for an infant using a duo or trio analysis, Athena analysts will conduct a retrospective analysis of only the infant's NewbornDx data. The purpose of this analysis is to determine if the same result obtained with a duo/trio could have been obtained with only the infant's sample. The analysts who conduct the retrospective analysis will not know the result that was returned for the subject. The retrospective infant-only analyses will occur on a periodic basis throughout the study.

RCIGM will also perform a retrospective infant-only analysis on all subjects utilizing either an Artificial Intelligence platform or by performing a manual analysis similar to Athena at the end of the study.

6.2 STUDY INTERVENTION COMPLIANCE

Adherence to the protocol regarding the study intervention will be assessed through the monitoring of critical processes as detailed in the Data and Safety Monitoring Plan (DSMP).

- Site procedure for maintaining accurate link of infant with study ID number
- Site procedure to ensure correct labeling of blood/DNA sample(s)
- Accurate and relevant Human Phenotype Ontology terms and clinical information submitted by the enrolling site to the laboratory for use in analysis of variants with phenotype
- Laboratory protocols adhering to CLIA/CAP/state guidelines
- Laboratory procedures for accurate matching of a result with the infant
- Laboratory procedures to ensure any secondary findings for the infant are only reported if the criteria pertaining to the infant as outlined in section 6.1.1 are met
- Laboratory procedures to ensure secondary findings for a biological parent are only reported if the criteria pertaining to a parent as outlined in section 6.1.1 are met
- Confirmation testing completed prior to reporting of pathogenic, likely pathogenic, or suspicious VUS results or the documentation of a provisional result exception
- Accurate dissemination of the report of results from the laboratories to the infant's EMR

6.3 CONCOMITANT THERAPY

There are no restrictions on concomitant medications, treatments or procedures allowed during the study. Medications and procedures will be captured during data collection.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/ WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

The study intervention is diagnostic tests. Discontinuation of the study intervention might occur if a clinical diagnosis is determined prior to the completion of NewbornDx or rWGS. In this case, the infant will be withdrawn from the study per section 7.2.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

An infant's parent/guardian or a biological parent can withdraw their own participation at any time and is not obligated to state the reason for withdrawal. However, the investigator should make a reasonable effort to ascertain the reason for withdrawal while fully respecting the parent/guardian's or biological parents' rights.

An investigator may discontinue or withdraw an infant from the study for the following reasons:

- A clinical diagnosis was determined prior to completing the genomic testing for the infant
- Withdrawal is requested by the NIH or DSMB
- If the infant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation (i.e. CMV testing that came back positive after enrollment)
- If the blood samples cannot be safely obtained from the infant
- The parent/guardian withdraws consent for the infant

The reason for discontinuation of the infant or biological parent will be recorded in the study database. An infant for whom the parent/guardian provided consent but who does not receive the study intervention may be replaced. An infant for whom the parent/guardian provided consent and who received the study intervention and was subsequently withdrawn from the study will not be replaced.

In the event of an infant death prior to the completion of genetic testing, the testing will continue to be done unless the parent/guardian indicates otherwise. There is a potential to provide information regarding a hereditary disorder for future children.

If an infant is withdrawn from the study, the data already collected on the infant may be used unless the parent/guardian indicates otherwise in writing.

The following actions will be taken at the local study site and laboratories as applicable after receiving a request to withdraw:

- Upon receipt of request to withdraw, the enrolling site will notify RCIGM and Athena (Quest) of withdrawal of the infant and/or the biological parent(s). Mount Sinai will also notify Sema4.

- The enrolling site or laboratories that are in possession of unused blood from the infant and/or the biological parent(s) will ensure blood samples are destroyed.
- Isolated DNA will be destroyed. The laboratories will permanently break any link between personal identifying information and sequencing data files from the infant and/or the biological parent(s).
- The RCIGM laboratory will remove the REDCap Infant Study ID from its ordering portal.
- If a back-up of the inventory database/informatics system is ever restored, then the laboratory should ensure that the relevant identifying records stored on the discarded samples log are again deleted from the records.
- The enrolling site must document reasons for withdrawal and record it in the REDCap database.
- The enrolling site will permanently break any link between personal identifying information and the anonymized records and data in REDCap by replacing protected health information (PHI) in the Master List of enrolled subject and REDCap Study Infant ID with a note that the infant has withdrawn.
- The enrolling site and laboratories must shred any hard copies of associated identifying information not required to be maintained for regulatory purposes.
- The enrolling site must retain the executed consent form and request for withdrawal in a separate, secure file of discontinued infants and redact any identifiers that link the infant to the data (such as infant's study ID number).
- The laboratories will certify that identifying links have been broken and as applicable, that biological material has been destroyed.
- Only anonymous data may be used for future research after consent has been revoked.

7.3 LOST TO FOLLOW-UP

An infant will be considered lost to follow-up if the enrolling site is unable to contact a parent/guardian at the time the infant turns 1 year CGA plus 2 months. In order to reduce the risk of lost to follow-up, study staff will have the parent/guardian(s) provide several methods of contact for themselves as well as a family member or friend who would know how to reach them if their contact information changed or are unreachable (refer to Follow-up Contact Information form). The enrolling site will attempt to contact the parent/guardian(s) monthly. Before an infant is deemed lost to follow-up, the enrolling site will make every effort to regain contact with the infant (multiple contacts and, if necessary, a certified letter to the infant's last known mailing address or local equivalent methods). These contact attempts should be documented in the infant's study file. If the infant continues to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up. It is permissible to contact the infant's primary care provider to obtain relevant information on the condition of the infant if the parent/guardian(s) are not available and they have given permission by signing the ICF and/or medical record release form.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCREENING & INFORMED CONSENT

Screening
Infants born with a suspected genetic disorder will be screened for all inclusion/ exclusion criteria. Potential infants will be identified by study personnel reviewing the daily census of applicable hospital locations and relevant medical records or by the clinical team. Study or clinical personnel will identify potential candidates based on the primary diagnosis, signs/symptoms, and CGA. The study team will discuss eligibility of the infant with the attending physician of record and if in agreement, the family will be approached for the study.

There is not a limit to how many infants can be enrolled at any one site. Infants who are already an inpatient at the time a site opens for enrollment may be approached for this study if they fulfill the inclusion/exclusion criteria. Those infants identified through the screening process will have the following data recorded on the Screening Log and/or entered into REDCap The Infant Study ID will be used to code screening data and is generated by REDCap consisting of a 4-digit number (1-digit site number and a 3-digit infant number).

Category	**Information from the medical record which may be recorded on a paper or electronic screening log kept securely at the enrolling site**	**Screening data that will be entered into REDCap if the infant is approached for study participation regardless of consent status**
Identifying Information	name, date of birth, medical record number	N/A
Screening Process	screening ID, date screened, location in hospital, whether or not the infant is eligible, if the family was approached for consent, date of approach, reason why an eligible infant was not approached	screening ID
Clinical Information	gestational age at birth, age at time of screening, clinical signs/symptoms and primary system involvement, clinical differential diagnosis, prenatal and postnatal clinical genetic testing and newborn screening results, list of genetic tests	gestational age at birth, age at time of screening, primary system(s) involvement related to patient nomination, sex of infant

	pending, newborn screening test results, sex of infant	
Consent Process	languages used during the consent process; if telephone consent was used; if clinical genetics was involved prior to the consent process; enrollment status; reason for declining study if voluntarily given	languages used during the consent process; if telephone consent was used; if clinical genetics was involved prior to the consent process; enrollment status; reason for declining study if voluntarily given
Demographics	type of health insurance, race and ethnicity of the biological mother and biological father (in order to represent the infant)	type of health insurance, race and ethnicity of the biological mother and biological father, 5-digit zip code

Identifying information from the screening log will be destroyed at the end of the study.

Note: for screened patients that have already been entered into REDCap under the current HIPAA authorization waiver by the IRB, we will first delete the primary signs/symptoms already captured in the database and then add the primary system involvement and zip code. The purpose of removing primary signs/symptoms and replacing it with system involvement is to reduce the risk of identification due to unique features once zip code is added.

Informed Consent
Consent by one parent/guardian is required for participation by the infant. Each biological parent will give permission to obtain and use his or her own blood sample and elect parental secondary findings. Written documentation of informed consent from the parent/guardian and/or biological parent(s) must be documented prior to the conduct of any study procedures. Refer to section 10.1.1 Process of Informed Consent.

If the parent/guardian provides consent for the infant to participate, study personnel at the enrolling site will:

- Review the ICF and ensure that it is properly completed
- Review whether or not the parent/guardian opted-in to receive secondary findings for the infant
- Review whether or not the biological parent(s) opted-in to receive secondary findings for themselves
- Provide the parent/guardian and the biological parents, as applicable, with a copy of the signed ICF
- Notify the attending physician of record of the parent/guardian's decision to participate
- Record that consent was obtained in the infant's medical record
- Place a copy of the signed ICF into the infant's medical record
- File the original signed ICF in the research file

- Complete screening log and the Demographics, Symptoms and Consent eCRFs in REDCap

If the parent/guardian refuses participation for the infant, study personnel will:

- Communicate refusal to relevant study or clinical personnel
- Complete screening log and the Demographics, Symptoms and Consent eCRFs in REDCap

8.2 ENROLLMENT

PI Assessment of whether or not an infant requires urgent testing
This protocol will prioritize testing of infants who are severely ill. Infants with the following conditions may qualify for the most rapid testing possible:

- Infant requires mechanical ventilation
- Infant is exhibiting severe neurological complications (i.e. uncontrolled seizures)
- Infant is hemodynamically unstable
- Site PI selects this option at his/her discretion

There is not a specific timeframe in which urgent testing must be returned; rather, the laboratory does its best to expedite testing. Refer to the “MOP about how to notify laboratories of urgent testing.

Enrollment in REDCap System
Complete the enrollment and urgent testing eCRFs in REDCap. The Infant Study ID and linking infant identifiers will be added to the secure site master enrollment log. The Infant Study ID will be used for all data entered into REDCap and in study files.

Ordering of Laboratory Tests, Collection and Shipment of Blood Samples
Three laboratories are involved in this protocol: Athena (Quest), RCIGM and Sema4.

1. Athena Diagnostics (a subsidiary of Quest Diagnostics) is performing the NewbornDx panel (infant and when available, the biological parents) and confirmation testing for pathogenic, likely pathogenic and suspicious VUS findings.
2. RCIGM is performing rWGS (infant and when available, the biological parents) and confirmation testing for pathogenic, likely pathogenic and suspicious VUS.
3. In some circumstances, Sema4 will be involved with DNA extraction and confirmation testing only for infants enrolled at Mount Sinai (refer to section 6.1.1).

Blood Sample Collection

Blood samples are collected as follows:

Who	Samples to Collect
Infant	• A minimum of two and maximum of five fully saturated dried blood spots (DBS) using study-provided Perkin Elmer 226 filter paper* • Two 0.5ml blood samples in study-provided EDTA tubes (one 0.5ml sample is acceptable, but two are preferred)
Mother	• Two 0.5ml blood samples in study-provided EDTA tubes (one 0.5ml sample is acceptable, but two are preferred) • One 2mL blood sample in a study-provided EDTA tube
Father	• Two 0.5ml blood samples in study-provided EDTA tubes (one 0.5ml sample is acceptable, but two are preferred) • One 2mL blood sample in a study-provided EDTA tube

*In circumstances in which an infant meets urgent criteria and where allowing the required 3-hour timeframe for a blood spot card to dry prior to shipping may adversely affect time to diagnosis and potential life-saving treatment, 1ml of whole blood in an EDTA tube may be obtained in lieu of the blood spot card and sent to Athena.

Refer to the MOP for detailed instructions about the timing and source of blood samples, collection procedures and documentation of the date/time of blood collection. If possible, collect blood from the infant at the time of routine clinical testing to minimize discomfort. If the clinical team does not feel it is safe to withdraw 1ml of blood from the infant, wait until just prior to the time a packed red blood cell transfusion is administered.

All blood samples will be obtained by qualified staff following the hospital guidelines and standard precautions. DNA extracted and held prior to enrollment may be used for this study (i.e. the clinical team obtained a sample prior to putting an infant on ECMO knowing an untainted sample may be needed later for clinical purposes).

Obtain whole blood samples from each biological parent near the time the samples are being collected from the infant. In the case of an assay failure or a damaged sample, an additional blood sample for the infant or a biological parent may be obtained and shipped to the laboratory.

Labeling of Blood Samples

Each sample should be labeled with name, date of birth and/or MRN, and sample ID number using permanent ink. Label each sample with name and date of birth and/or MRN at the time of sample collection. Add the sample ID number for each sample after you complete the laboratory requisition forms. The sample ID numbers for rWGS are generated by the RCIGM ordering portal at the time the online requisition is completed. The sample ID numbers for NewbornDx will be generated manually by the site as instructed in the MOP.

Blood Sample Storage Conditions until Shipment

Keep the blood samples refrigerated at 4°C until the time of shipment (up to 5 days). Do not freeze. The DBS may be kept at room temperature until shipment.

Order NewbornDx and rWGS

Complete the requisitions for each laboratory as follows and according to the MOP:

The site shipping samples	Requisitions to complete	Process
All sites (except Mount Sinai)	Athena Diagnostics	Complete the paper Athena requisition according to the MOP, keep a copy in the infant's research file and include it when shipping samples to Athena.
	RCIGM	Login to the HIPAA-compliant RCIGM ordering portal and complete the online test requisition form according to the instructions in the MOP. The RCIGM ordering portal will 1) assign a RCIGM Portal Case ID and 2) generate a RCIGM sample ID code for each sample. Print the requisition form, keep a copy in the infant's research file and include it when shipping samples to RCIGM.
Mount Sinai	Athena Diagnostics	Complete the paper Athena requisition according to the MOP, keep a copy in the infant's research file and include it when shipping samples to Athena.
	RCIGM	Login to the HIPAA-compliant RCIGM ordering portal and complete the online test requisition form according to the instructions in the MOP. The RCIGM ordering portal will 1) assign a RCIGM case ID and 2) generate a RCIGM sample ID code for each sample. Print the requisition form, keep a copy in the infant's research file and include it when providing samples to Sema4 (don't ship whole blood samples to RCIGM).
	Sema4	Complete the paper-based Sema4 requisition, keep a copy in the infant's research file and include both the Sema4 requisition and the RCIGM requisition when providing samples to Sema4.
Sema4	RCIGM	Include the RCIGM requisition completed by and received from Mount Sinai when shipping the DNA samples to RCIGM.

Be sure to indicate in the appropriate fields on all requisitions whether or not the parent/guardian opted-in to receive secondary findings for the infant and if each biological parent opted-in for their own secondary findings.

Provision of Clinical Information to the Laboratories

HPO Terms will be provided by the site to the laboratories to inform the data interpretation of the genomic sequencing (refer to section 6.1.1, Phenotype Extraction and instructions in the MOP). It is the responsibility of the site to ensure the infant's clinical condition is monitored while NewbornDx and rWGS are in process. Refer to the MOP about how to communicate any changes (either positive or negative) in the phenotype of the infant after the HPO terms are sent.

Laboratory Notification of Urgent Testing

If the site PI determines an infant requires urgent testing (refer to urgent testing criteria above), the site should order an "ultra-rapid" WGS test in the RCIGM portal and contact Athena at the time of sample shipment to inform them of urgent testing. Mount Sinai should contact Sema4 to extract the DNA and ship it to RCIGM as quickly as possible. How to notify the laboratories of urgent testing is detailed in the MOP.

Blood Sample Shipment

The shipping address, shipping conditions and when to delay shipment of the infant's sample to obtain parent samples is described in the MOP. Only laboratory personnel with International Air Transport Association (IATA) certification should package/ship specimens. Ship samples overnight delivery. RCIGM must receive the blood samples by **10AM PST** to ensure it is on that day's sequencing run. Athena Diagnostics does not accept samples on Sundays. RCIGM will receive samples on Sunday if the ordering provider determines that the patient is critically ill and requires results as fast as possible; contact RCIGM prior to shipping samples.

Receiving Lab	Enrolling Site	What the Enrolling Sites Ship
Athena	All Sites	○ Infant: minimum of 2 Dried Blood Spots on one DBS Card ○ Mother: 2ml of whole blood in one EDTA tube ○ Father: 2ml of whole blood in one EDTA tube ○ Athena Requisition Form
RCIGM	All sites except Mount Sinai	○ Infant: 0.5ml whole blood in each of two EDTA tubes ○ Mother: 0.5ml whole blood in each of two EDTA tubes ○ Father: 0.5ml whole blood in each of two EDTA tubes ○ RCIGM Requisition Form
Sema4*	Only Mount Sinai	○ Infant: 0.5ml whole blood in each of two EDTA tubes ○ Mother: 0.5ml whole blood in each of two EDTA tubes ○ Father: 0.5ml whole blood in each of two EDTA tubes ○ Sema4 Requisition Form ○ RCIGM Requisition Form

*Sema4 will isolate DNA from the blood samples, ship DNA aliquots for the infant and each biological parent to RCIGM and keep an aliquot at Mount Sinai for possible confirmation testing.

Tracking of Samples
When the samples are shipped, the site research team should retain the tracking number for each shipment to confirm the package arrived at the applicable laboratory. Sites should send the courier name and tracking number applicable to Athena (Quest) and RCIGM via the email addresses provided in the MOP.

Data Collection and Entry into the REDCap System
The following information from the infant's medical record entered into REDCap by staff at each enrolling site. Enrolling sites will have access to data entered into REDCap for infants at their own site. The study-wide co-PIs (Davis/Maron) and monitoring staff will have access to all data in REDCap.

Refer to the detailed grid of Data eCRFs in section 1.3 Schedule of Activities and the eCRF Instructions. Information collected and entered into the REDCap database for enrolled infants will include:

- identifiers (date of birth, date of hospital admission, dates of tests/procedures, parent email address if the parent wishes to complete follow-up surveys online – this email is not released or used outside of this study and will be removed when de-identifying the dataset)
- birth information (mode of delivery, birth weight, APGAR score, newborn screening results, pregnancy complications)
- phenotypic information (clinical symptoms, clinical presentations, HPO terms)
- clinical information (diagnoses, laboratory and imaging test results including clinical genetic testing results)
- clinical genetic tests that would have been done if this research study was not available
- genetic testing results as a result of study participation
- any actions because of the research genetic test results
- treatments (surgical procedures and medications)
- hospital billing information (CPT codes, billing data)
- medical visit, emergency room, hospitalization, procedures, treatments post discharge home
- quality-of-life assessments
- date and cause of death, if applicable
- adverse events

Data for this study will be collected at the following time points: at enrollment, following return of results, at time of an adverse event, at transfer, discharge home, withdrawal, or death and then monthly after discharge until the infant turns one-year CGA.

Upload of Source Documents to the REDCap System
Scanned source documents will be uploaded to the REDCap system for remote monitoring. Copies of medical records or source documents should be redacted of name and MRN or other account numbers, coded with the Infant Study ID, scanned to a PDF file and uploaded to REDCap.

Genomic Sequencing Results

Please refer to Section 6.1 Description of Study Intervention for details regarding the genomic sequencing methods that will be completed. Results of the infant's genomic testing will be shared with the research team, clinical team, the parent/guardian(s), and placed in the infant's medical record as follows:

Type of result	Result reported by laboratory	Action at site
Provisional Positive Result	Diagnostic findings related to phenotype - pathogenic variant(s) in genes interpreted to be responsible for, or contributing to, the infant's phenotype will be reported (classification of pathogenic or likely pathogenic). A provisional result means the result has not been confirmed. A provisional result is only given if waiting for confirmation testing would put the infant at risk for significant morbidity or mortality (a treatment is available and a delay would cause irreversible harm). For all provisional results, confirmation testing will subsequently be performed.	The enrolling site will receive management guidance by the laboratory and consult with their clinical genetics team. Provisional results will be entered in the infant's medical record under a research note. The research note will outline what provisional results were reported. Provisional results may guide the implementation of an intervention for the infant. Following clinical confirmation of results, a clinical report of the final result will be placed in the medical record. In the event that the positive finding cannot be confirmed, a research note will be added to the medical record to facilitate communication among multiple specialty services.
Confirmed Positive Result	Diagnostic findings related to phenotype - pathogenic variant(s) in genes interpreted to be responsible for, or contributing to, the infant's phenotype will be reported (classification of pathogenic or likely pathogenic). The result has been confirmed (for example with Sanger or qPCR). All positive results will be confirmed prior to return to the enrolling site with the exception of a provisional result.	Confirmed positive results will yield a clinical diagnosis, a referral to clinical genetics and reported in the medical record. Results may guide the implementation of an intervention for the infant.
Confirmed Variant of Unknown Significance (VUS)	A variant for which the clinical significance is unknown. The variant detected may or may not explain the infant's current clinical symptoms. The variant has been	Suspicious VUS reported by the laboratories will be reported in the medical record. In the future, a variant may become known to be significant, and the parent/

Type of result	Result reported by laboratory	Action at site
	confirmed. Only suspicious VUS (where the phenotypic fit is very good and/or functional confirmatory tests are readily available and/or the results are actionable) will be reported by the laboratory.	guardian(s) of the infant will have this information to provide to their personal physician.
Negative Result	The result that pathogenic variants associated with the infant's clinical phenotype were not detected (classification likely benign or benign).	Negative results will be reported to the medical record in accordance with standard care for clinical laboratories to reflect the clinical testing ordered.
Secondary/ Incidental Finding for the Infant	Variant(s) not thought to be related to the infant's current symptoms but still important for the person's health will be reported if 1) the parent/guardian opted-in to receive additional results for the infant, 2) the onset of the disorder may occur in childhood, and 3) at least one of the following: a) the disorder is medically actionable (available treatment to cure or ameliorate symptoms or preventative measure), or b) The disorder is of a gene on the "ACMG list" of genes recommended for reporting of secondary findings at the time of testing.	Upon receipt of a report with a secondary/incidental finding for the infant, the site will verify on the signed ICF that the parent/guardian(s) opted-in to receive additional results for the infant. The result will be given at the same time as a primary finding for the infant.
Secondary/ Incidental Finding for a Biological Parent	If a duo or trio is done, variant(s) not thought to be related to the infant's current symptoms but still important for the biological parent's health will be reported if 1) the biological parent opted-in during the informed consent process to receive additional results and 2) the variant is of one of the 59 genes recommended by the ACMG to report back as secondary findings. Secondary findings for a biological parent will	Upon receipt of a report with a secondary/incidental finding for the biological parent, the site will verify on the signed ICF that the biological parents opted-in to receive additional results. The result will be given to the biological parents by the enrolling site PI, study geneticist or genetics counselor and a referral to clinical genetics will be made.

Type of result	Result reported by laboratory	Action at site
	be returned in a separate report from the report issued for the infant and the biological parent report will not be put in the infant's medical record. The report of a secondary finding for a biological parent will be issued to the enrolling site PI.	

Clinical Laboratory Report

Standard clinical laboratory reports of results will be issued from Athena Diagnostics and RCIGM. The report of results will include: test result [positive (diagnosis), negative, variant of unknown significance], sequence variants detected (gene and transcript, chromosome, genomic coordinates, variant, confirmation status), variant interpretation, gene information, references, recommendations, test methodology, test limitations, regulatory disclosures, laboratory contact information, CLIA #, CAP #. Exception: Until conditional New York State CLIA certification and rWGS approval is obtained, RCIGM will issue a research report for subjects from Mount Sinai. For results that are confirmed by Sema4, Sema4 will issue a standard clinical laboratory report.

Raw Sequencing Data

Under California law (Assembly Bill No. 375), subjects have the right to obtain their own sequencing data from for-profit companies. Athena (Quest) Diagnostics is required to make sequencing data available upon request if paid a data recovery fee by the subject. This information is included in the informed consent form. While not mandated per law, raw data can be downloaded from the RCIGM portal for 60 days after the results are returned. After this time, sequencing data can be obtained by request with a turn-around time of approximately 1 week.

At the end of the study, VUS may be re-analyzed to see if gains in the understanding of genotype and phenotype correlation have occurred over the length of the study which changes the infant's results (i.e. a VUS becomes positive). In such a case, the site will attempt to notify the parent/guardian(s).

Future Use of Specimens and Sequencing Data

DNA may be stored for further testing if necessary for the management of the infant. Stored DNA will be destroyed at the conclusion of the study per section 10.1.4, Future Use of Stored Specimens and Data.

Process for the laboratory to return results to the research team and the medical record

Notification of a Provisional Result:
If any of the laboratories have a positive result requiring provisional reporting, the laboratory must:

1. Call three parties: 1) the attending physician taking care of the infant, 2) the site PI and 3) study-wide co-PI Dr. Jill Maron. The lab will use the contact information provided by the site on the requisition to reach the attending physician of record. Therefore, the site will determine the best number (e.g. the clinical unit phone number where the infant is admitted or the beeper/phone that the NICU fellow holds 24/7). The phone numbers for each site PI and Dr. Maron are listed in the MOP. Provide verbal provisional result and management guidance with read back. The notifying laboratory will document the person to whom the result is reported and the date and time of notification (refer to the form, "Documentation of Verbal Result Returned").
2. Send an encrypted email of a PDF of the provisional result report to 1) the physician of record, 2) the site PI/research team, 3) the study-wide Co-PIs and project manager. The email addresses to reach the physician of record should be obtained during the verbal report. The RCIGM portal will be configured to automatically trigger a copy of the email alert to the site study team and the study-wide Co-PIs. Athena (Quest) will refer to the email addresses for each site PI and Dr. Maron listed in the MOP but these should also be included on the requisitions. Refer to the MOP for contact information. The provisional result will not be digitally faxed directly into the EMR.

The site will enter provisional results in the infant's medical record under a research note. The research note will outline what provisional results were reported. Following clinical confirmation of results, a final clinical report will be placed in the medical record. In the event that the positive finding cannot be confirmed, a research note will be added to the medical record to facilitate communication among multiple specialty services.

Notification by the Laboratory of a Confirmed Positive Result, Negative Result or Suspicious VUS:
The laboratory must:

1. Send an encrypted email of a PDF of the report to 1) the site research team and 2) the study-wide Co-PIs and project manager. Refer to the MOP for contact information.
2. In the case of 1) a confirmed positive result or 2) a final result that has the potential for immediate implications for the infant's management (i.e. provisional result that is not confirmed), call the attending physician of record caring for the infant. Provide verbal results with read back. The notifying laboratory will document the person to whom the result was reported, and the date and time of notification (refer to the form, "Documentation of Verbal Result Returned"). After the site is contacted, the laboratory must call the site PI and Dr. Maron.

3. During the course of this study, we intend to move to a process in which the laboratory issues the report directly to the infant's EMR by digital fax. For infants from Mount Sinai, the RCIGM report will be sent to the Mount Sinai PI, not the infant's EMR, for input into the subject's research file. After completion of confirmation testing by Sema4, if applicable, the Sema4 CLIA-certified report will be sent to the infant's EMR.

Notification by the Laboratory of Secondary Findings

1. If the parent/guardian(s) opted-in to receive secondary findings for the infant, the laboratory will report secondary findings as described in section 6.1.1 in the return of results for the infant.
2. If a biological parent opted-in to receive secondary findings for him or herself, the laboratory will return secondary findings for the individual biological parent as described in section 6.1.1 in a separate report from the test result for the infant. This report will not be sent to the infant's EMR. It will be sent to the site PI.

Process for returning results to the medical record

It is intended for the report of results to be sent Athena and RCIGM directly to the infant's EMR via digital fax; however, the enrolling site may need to manually deposit the report in the medical record until that process is completed. The enrolling site is responsible for ensuring that the clinical laboratory reports appear in the infant's medical record. If the final results are discordant, all reports will still be placed in the medical record and a note should document how the results will be used in clinical care. In the case of infants enrolled at Mount Sinai, only results by a New York State CLIA certified lab of a New York State approved or conditionally approved test will be put in the infant's medical record.

Clinical genetics and related sub-specialty notes will be placed in the medical record per SOC.

Adjudication of Discordant Results

If the final results of NewbornDx and rWGS or the confirmation test results done at Sema4 are discordant, adjudication processes, as outlined in the MOP, will be followed. If results are discordant due to variant classification, the laboratories will each provide the evidence supporting their result and variant classification. This summary will be given to the enrolling site PI to share with the clinical team; a subsequent phone call with the labs may be arranged upon request from the site. The clinical team caring for the infant is in the best position to determine if one result best explains the phenotype of the infant and their view will be captured in the study data. Additionally, a subset of the study geneticists on the steering committee who are independent of the labs will review the redacted lab reports and lab summaries and provide what they think is the most accurate result for an additional study endpoint. Senior lab personnel are available for questions by the steering committee. The opinion of the steering committee members may be shared with the enrolling site upon the site's request. Regardless of whether or not results between the tests are concordant, both the NewbornDx report and the rWGS

report will be put in the infant's medical record. A note should be placed in the medical record to document how the results will be used in clinical care.

Communication with the Physician of Record and the Infant's Parent/Guardian(s)
The site should wait until results from both NewbornDx and rWGS are returned before speaking with the parent/guardian about the results as long as waiting would not harm the infant (i.e. the result is not provisional or the infant does not require urgent testing).

Coordination Between Study Team and Physicians of Record re: Results	Qualifications of Personnel providing Genetic Results to Parents/Guardians	Qualifications of Personnel providing ongoing related information to parents/guardians	Providing Results to Parents/Guardians when an Infant is Discharged Home Prior to Return of Results
The site PI/sub-I will speak with the physician of record and suitable subspecialists (for example, genetics, neurology, cardiology, etc.) at the time each test result is returned in order to review results and formulate a plan prior to returning a result to the parent/guardian. The PI and/or sub-I at all sites are neonatologists or geneticists. If the patient is discharged home prior to the return of results, the primary pediatrician should be contacted along with hospital clinical and research personnel.	Results will be disclosed to the family with as many members of the clinical team caring for the patient present as possible. At a minimum, each site will include one or more of the following: attending clinical geneticist, attending physician of record, attending neonatologist or geneticist who is a member of the study team. Additional attending sub-specialists relevant to the particular genetic condition and genetic counselors may also participate in providing results to families. If clinical practice at a particular site is for licensed genetic counselors to return results without an attending physician as described above, the licensed genetic counselor may do so.	Recommendations to the family about changes in management or further genetic testing will be addressed by the physician of record as well as clinical genetics and other sub-specialties per standard-of-care. If a genetic problem exists that can be treated but does not need to be treated in the neonatal period, a referral to outpatient clinical genetics will be made.	It is strongly recommended that positive results or a VUS be disclosed in-person followed by a referral to a clinical geneticist. Depending on the need for timely action based on the results, it may be appropriate to return the result at the time of the next outpatient clinical genetics or related subspecialist appointment or to ask the family to come into the site to meet with the site PI to hear about the study results. Under extraordinary circumstances (where the family lives hours away and/or has moved out of state), diagnostic or VUS results may be given by phone along with a clinical genetics referral. It is acceptable to convey negative/no finding results by phone with an offer for follow up genetic counseling visit.

Communication with a Biological Parent about a Parent Secondary Finding
Upon the receipt of a report with a secondary finding for a biological parent by the site PI, the site will verify on the signed ICF that the biological parent opted-in to receive secondary findings. The result will be given to the biological parent by the enrolling site PI, study geneticist or genetics counselor and a referral to clinical genetics will be made. Some sites may opt to create a medical record for each biological parent and scan the consent and individual parental secondary findings to that medical record.

Genomic Sequencing Clinical Utility Survey
At discharge home or within one month after the return of results for infants who discharge home prior to the return of results, the physician of record at the time the results are returned will be asked to complete a brief survey regarding the clinical utility of the genomic testing. The one physician under whom the infant is admitted at the time results are received will receive the survey (i.e. neonatologist if the infant is in the NICU, pediatric critical care attending if the infant is in the PICU). The survey will be sent via email or a paper copy handed to the physician. The goal of the survey is to identify what changes were made to the immediate management of the infant as a result of testing as well as the general utility of the diagnosis. If the infant is discharged (home or death) prior to return of results, the Clinical Utility Survey should be completed by the clinical geneticist, most relevant subspecialist or by the site PI after speaking with the pediatrician (if baby was discharged home).

Parent/Guardian Contact Information, Medical Record Release and Diaries at Transfer/Discharge Home
The parent/guardian(s) will fill out a contact information form with multiple methods of contact to ensure we are able to reach the parent/guardian or primary care provider until the infant is 1 year CGA. A quality of life (QoL) instrument (The Optum™ SF-12v2® Health Survey) and the Child Visual Analog Scale will be administered.

Billing Information
At transfer to another facility and/or discharge home, the enrolling site research staff will obtain the final hospital costs and charges, and all physician CPT codes, for the infant's hospitalization.

Parent/Guardian Interview and Medical Record Acquisition
Following transfer of facility or discharge home, study staff will contact one parent/guardian monthly to assess how the infant is progressing until 1 year CGA. The parent/guardian will be asked about any new testing, diagnosis, medications, medical visits or hospitalizations, and any missed days of work due to their child's disorder. Ideally, the parent will receive a link via email to complete the monthly parent survey online in the REDCap system; however, the parent/guardian may be contacted by e-mail or phone per the parent/guardian's preference. Self-reported QoL information will be collected from a parent/guardian at 3-month intervals to infer the impact on the family of any prolonged illness. The follow-up surveys will be translated into Spanish. In the case of a parent/guardian who does not read English or Spanish, the follow-up contact will need to be made with an interpreter by phone. Upon request by the health

economics analysts and as permitted in the ICF, medical records and billing information from any hospitalizations and any outpatient health care utilization may be obtained from the provider institutions to complement parent/guardian self-report.

8.3 SAFETY AND OTHER ASSESSMENTS

Enrolling sites must actively monitor, identify and assess adverse events (AE) in the study. Identification of AEs will be based on review of the medical record, observation by study staff and by report during the parent survey/interviews. For each AE, the investigator will determination whether the adverse event is serious, provide the duration (start and end dates), the severity grade (mild, moderate, severe), the relationship to the study testing (unlikely, possibly, and probably), action(s) taken, and the outcome. The AE assessment must be documented and retained with study files. The Investigator's signature and date on the source document that supports the causality noted on the AE form ensures that a medically qualified assessment of causality was done.

The laboratories conducting genetic sequencing for this study fall under the CLIA/CAP regulations. They continually monitor for errors and non-conforming events and will notify the study-wide PIs via phone/email of an error/event that affected a result for the infant. They will also notify the study-wide PIs if a data breach were to occur. In these instances, the study-wide PI would file an adverse event, and notify the DSMB, IRB, and NCATS as required. The laboratories are not expected to report adverse events as described in sections 8.3 and 8.4.

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.4.1 DEFINITION OF ADVERSE EVENTS (AE)

An Adverse Event for this study will only include the following:

- An untoward medical occurrence related to obtaining the study blood samples
- If findings for the infant thought to be secondary at the time results are reported are given to the research team, clinician or parent/guardian when the parent/guardian did not opt-in to receive secondary findings for their infant
- If secondary findings for a parent are reported to the research team, clinician or parent when the parent did not opt-in to receive parental secondary findings
- Intervention based on inaccurate result returned (testing error; provisional result not confirmed)
- Intervention based on wrong result returned (human error, result not accurately matched up with the correct infant)
- Untoward event related to an intervention initiated due to the genomic testing results (intervention that would not have been initiated without the genomic testing results)
- Loss of confidentiality related to study participation

- Any new untoward medical occurrence related to study participation or the genetic sequencing results
- A pre-existing condition that worsens in terms of frequency or intensity and the worsening is due to study participation/genetic sequencing results
- The infant dies due to error because the infant was enrolled in this study (erroneous result or misidentification)

Events not reported as Adverse Events for this study:

- A pre-existing condition at the time of enrollment
- A pre-existing condition that worsens in terms of frequency or intensity but the worsening is not related to study participation/genetic sequencing
- A new untoward medical occurrence that is not related to study participation/genetic sequencing results and does not result in death
- Death due to natural causes before genetic testing results are returned (i.e. prematurity)
- Death due to parent/guardian wishes to withdraw life sustaining treatment if an infant is determined to have a lethal condition by genetic testing

8.4.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require more prolonged hospitalization may be considered serious when they may jeopardize the infant and require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Events that are not related to study participation or genomic sequencing will not be captured as an SAE since the definition of an adverse event for this study requires a relation to genetic testing (rather than the underlying disorder).

8.4.3 CLASSIFICATION OF AN ADVERSE EVENT

The PI or another licensed clinician who is a study member authorized on the delegation log will classify adverse events.

8.4.3.1 SEVERITY OF EVENT

The investigator should use the following definitions when assessing intensity of an adverse event:

- Mild: Infant or a biological parent has minor findings but tolerates them well, and no or minimal intervention required

- Moderate: Infant or a biological parent experiences enough symptoms or findings to require intervention
- Severe: Infant or a biological parent experiences symptoms or findings that require significant intervention

8.4.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to the genomic sequencing assessed. The investigator will examine and evaluate the participant based on temporal relationship and his/her clinical judgment.

The criteria below are intended as guidelines to assist the Investigator in assessing the likelihood of a relationship between the genomic sequencing testing and the adverse event. The greater the correlation with the components and their respective elements, the more likely the genomic sequencing testing caused the adverse event.

- *Exposure*: Is there evidence that the infant or biological parent had the testing performed such as: reliable history, patient records, assays run in applicable laboratories and results exist?
- *Time Course*: Did the AE follow in a reasonable temporal sequence from performance of the testing? Is the time of onset of the AE compatible with the testing being performed?
- *Likely Cause*: Is the AE not reasonably explained by another etiology such as an underlying disorder or other host or environmental factors?

The following scale of criteria may be used as a guidance (not all criteria must be present in order to be indicative of a relationship to study genomic sequencing).

Probably related to study genomic sequencing	• There is evidence the infant or a biological parent had the genomic testing performed • The temporal sequence of the AE onset relative to genomic testing is reasonable • The AE is more likely explained by the genomic testing than by another cause
Possibly related to study genomic sequencing	• There is evidence the infant or a biological parent had the genomic testing performed • The temporal sequence of the AE onset relative to genomic testing is reasonable • The AE could have been due to another equally likely cause
Unlikely related to study genomic sequencing	• There is evidence the infant or a biological parent had the genomic testing performed • There is no temporal relationship to genomic testing • There is another more likely cause of the AE

8.4.3.3 EXPECTEDNESS

The investigator will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention. An AE is considered unexpected if it is not consistent with the risk information described in the protocol.

8.4.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

AEs must be actively identified from the signing of the ICF until the infant reaches one year CGA. AEs must be followed until an outcome is known. All events that cannot be resolved by 30 days after the final assessment at 1 year CGA will be considered resolved by convention and entered in the REDCap system.

8.4.5 ADVERSE EVENT REPORTING

Reporting of non-serious AEs must occur using the Adverse Event eCRF in the REDCap system according to the following:

Event	Timeframe for Site to Report	Timeframe for Study PI to Report
AE, not serious	Site enters AE into EDC within 7 business days of identification.	The study PI reports to the DSMB every 6 months and in the annual progress report to NCATS.

8.4.6 SERIOUS ADVERSE EVENT REPORTING

Reporting of SAEs must occur using the adverse event eCRF in the REDCap system according to the following:

Event	Timeframe for Site to Report	Timeframe for Study-wide PI to Report
SAE	Site reports to the study-wide PI within 24 hours of learning about the event.	The study-wide PI will alert the DSMB chair within 24 hours of learning about the event. If the SAE is also an unanticipated problem, the study-wide PI will also report to the central IRB and NCATS within 24 hours of learning about the event.

To ensure accurate clinical trial reporting and correct interpretation of safety signals, each death will be examined to determine cause. This may be difficult in the preterm infant population as there are often multiple events which contribute to death. For the

purposes of this clinical trial, the investigator will attribute the cause of death to the AE which had the greatest medical contribution to death, regardless of whether or not the event was the most proximal. For example, if an infant has a large intraventricular hemorrhage that leads to seizures and death, the death should be attributed to intraventricular hemorrhage, not seizures. The investigator should utilize all available information when attributing cause of death. Autopsy information should be utilized where available to amend the adverse event report if needed. It is recognized that the death certificate may not reflect clinical trial procedures and thus may not match the cause of death attributed by the investigator. In the rare situation for deaths in which no single AE represents the greatest medical contribution to death, the adverse event is listed as "Death not associated with an AE term" and one of the following causes must be selected: 1) death not otherwise specified (i.e. failure to thrive), 2) extreme prematurity, 3) multi-organ failure, and 4) sudden death (no documentation of cause, includes Sudden Infant Death Syndrome). For cases in which the cause of death is unclear, the final determination as to which AE or organ system the death should be attributed will be determined by the DSMB.

8.4.7 REPORTING EVENTS TO PARTICIPANTS

The parent/guardian of an infant will be told of AEs/SAEs in the course of the infant's clinical care (i.e. untoward event related to treatment initiated from sequencing results) or in the instance of an inaccurate or wrong result returned. If the risk of participating in the study changes, re-consent of the parent/guardian or of a biological parent will occur at the discretion of the IRB and DSMB.

8.5 UNANTICIPATED PROBLEMS

8.5.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

"Unanticipated Problems Involving Risks to Subjects or Others" ("UPIRSO"). An event is considered an UPIRSO when it meets **all** of the following criteria:

(1) It is unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the population being studied;

Unexpected events could be either medical or non-medical events.

(2) It is related or possibly related to participation in the research (i.e. there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research);

and,

(3) It places subjects or others [e.g. study team members or relatives of a subject] at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized

8.5.2 UNANTICIPATED PROBLEM REPORTING

Certain types of events (as defined below) and including unanticipated problems involving risks to subjects or others must be promptly reported to the IRB:

Event	Timeframe for Site to Report	Timeframe for Study PI to Report
Certain types of events (as defined below)	Sites much report the event to the study-wide co-PIs as soon as possible but no later than 8 working days after discovery or in the case of a death within 24 hours of learning about the event.	The study PI will alert the central IRB, DSMB Chair, and NIH as soon as possible after the event is discovered by the site, but in all cases within 10 working days after discovery of the event by the site. Reportable deaths, as described in this section, must be reported as soon as possible but in all cases within 72 hours after discovery by the site.

The following “certain types of events” must be promptly reported to the IRB:

A. UPIRSO (refer to definition in section 8.4.1)

B. POTENTIAL SERIOUS OR CONTINUING NON-COMPLIANCE:

Non-Compliance is defined by the Organization as the failure to follow the research protocol, federal, state, or local laws or regulations governing human subjects research, institutional policies, or the requirements or determinations of the IRB. Only incidents that may qualify as serious or continuing non-compliance must be promptly reported:

(1) Serious Non-compliance is defined by the Organization as non-compliance that either (a) significantly harms or poses an increased risk of significant harm to subjects or others, or (b) significantly compromises the rights and welfare of the subjects or the integrity of the Organization’s human research protection program.

(2) Continuing Non-compliance is defined by the Organization as a pattern of non-compliance that significantly compromises the scientific integrity of the study or the rights and welfare of the subjects or the integrity of the Organization’s human research protection program. When applying this definition, particular consideration may be given by the IRB to activity that recurs after a previous report has been evaluated by the IRB and corrective action has been instituted.

C. OTHER EVENTS THAT REQUIRE PROMPT REPORTING:

In addition to the above, investigators must also promptly report the following:.

- Potential Breaches of Confidentiality: Any unauthorized disclosure of subject's personally identifiable information. Please Note: Potential breaches of confidentiality that involve protected health information (PHI) must also be reported promptly to the HIPAA Privacy Officer. Please see guidance for further detail.
- Incarceration of a parent participating in this study
- Unresolved Subject Complaints: Complaints of subjects when the complaint indicates unexpected risks or cannot be resolved by the research team.
- Other events: There may be other events that should be promptly reportable to the IRB.

Events that do not meet the above criteria should be summarized and reported to the IRB at the time of continuing review.

8.5.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Refer to section 8.3.7

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

The analysis plan is descriptive. There will be no statistical hypothesis testing.

9.2 SAMPLE SIZE DETERMINATION

The sample proportion and 95% CI for the binary outcomes of NewbornDx and rWGS will be calculated. The table below shows the 95% confidence limits of the estimated proportion for a range of proportions, assuming the sample size is 400 completed infants. For example, if the sample proportion is 0.05 for disorders detected by rWGS but not NewbornDx, the 95% CI will be 0.03 to 0.07. Since the distribution of time to diagnosis is unknown, it is difficult to speculate as to the width of the confidence interval for the mean or median. However, 40% to 60% (160 to 240 infants) are expected to have a genetic diagnosis which is a sufficient number for a precise estimate.

Table: Confidence Interval Limits with 400 infants

Proportion	Lower Limit	Upper Limit
0.05	0.03	0.07
0.10	0.07	0.13
0.20	0.16	0.24
0.30	0.25	0.35
0.40	0.35	0.45
0.50	0.45	0.55

9.3 POPULATIONS FOR ANALYSES

- ICF Dataset: All families who were approached for study participation
- Intention-to-Treat (ITT) Dataset: all consented infants
- Modified Intention-to-Treat Dataset: all consented infants for whom both NewbornDx and rWGS genomic sequencing results were returned
- Per-Protocol Dataset: all consented infants who received both NewbornDx and rWGS (modified intention-to-treat dataset) and completed the study period for the analysis of the primary and secondary objectives without clinically important protocol deviations. Clinically important protocol deviations will be identified and documented prior to the database lock.
- Time to Molecular Diagnosis Dataset: The Modified Intention-to-Treat Analysis Dataset excluding the infants who did not have a confirmed positive result
- Urgent Testing Dataset: All infants who required urgent testing per section 8.1

- Safety Analysis Dataset: All infants for whom blood specimens were obtained or data collected

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

All analyses will be descriptive. We will calculate the proportion and 95% CI among enrolled infants with confirmed genetic disorders detected by NewbornDx, by rWGS, by NewbornDx but not rWGS, and by rWGS but not NewbornDx. We will also calculate the 95% CI for the ratio of the proportion detected by NewbornDx relative to the proportion detected by rWGS[69]. Although detection by SOC is an exclusion criterion, some infants will receive a diagnosis by SOC after enrollment. We will therefore also calculate the proportion and 95% CI of those receiving a diagnosis by SOC prior to the time that results from NewbornDx or rWGS become available. We will calculate the proportions and CIs for enrollees with no confirmed diagnosis of a genetic disorder. These will be calculated at discharge and at 1 year CGA. We will calculate the proportion of infants with conditions detected by NewbornDx that were not listed as part of the differential diagnosis at enrollment. The same calculation will be performed for rWGS. We will calculate the positive predictive value (PPV) of NewbornDx and rWGS (i.e. the proportion of positives that are confirmed by Sanger or PCR).

Because a “gold standard” does not currently exist, it will not be possible to calculate the negative predictive value (NPV), sensitivity, or specificity of NewbornDx or rWGS. We will calculate the proportion for which there was clinical utility from genomic testing (change in drug treatment, surgical treatment, imaging, subspecialty consult, diet, other treatment, and redirection of care and/or withdrawal of life sustaining treatment). We will also calculate the proportion of testing where trios were ultimately required to confirm a diagnosis for rWGS) and if this approach increased the number with a confirmed diagnosis. Kaplan-Meier (K-M) curves will be used to estimate the time from enrollment to molecular diagnosis (separately for NewbornDx and rWGS) including all enrolled infants and excluding infants without a diagnosis of a genetic disorder and those diagnosed by SOC before genomic results are returned. The analysis will count as positive only those diagnoses that are eventually confirmed. We will also use K-M to estimate age at diagnosis and survival. A model will be developed to predict, using information available at the start of symptoms, whether the NewbornDx ultimately yields a confirmed and actionable diagnosis. Such a model could help to further target NewbornDx to the population that would benefit most from it, especially if the model discrimination is high (c-statistic greater than .90). The model will be validated using bootstrap validation.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

Primary Endpoints	Definition	Scale and measure	Populations for which the analysis will be conducted
A confirmed positive result detected by NewbornDx	If NewbornDx and the confirmatory test have the same positive finding	Binary: Yes/No; single endpoint	Modified Intention-to-Treat Dataset
A confirmed positive result detected by rWGS	If rWGS and the confirmatory test have the same positive finding	Binary: Yes/No; single endpoint	Modified Intention-to-Treat Dataset
Time to a positive result by NewbornDx *	Among those who have a confirmed positive result at one year CGA by NewbornDx, rWGS, or another genetic test, comparison of date/time of sample collection to date/time of completion of NewbornDx prior to confirmation testing	Interval: Hours; single endpoint	Time to Molecular Diagnosis Dataset
Time to a positive result by rWGS*	Among those who have a confirmed positive result at one year CGA by NewbornDx, rWGS, or another genetic test, comparison of date/time of sample collection to date/time of completion of rWGS prior to confirmation testing	Interval: Hours; single endpoint	Time to Molecular Diagnosis Dataset
Time to a result by NewbornDx*	Among all babies tested, comparison of date/time of sample collection to date/time of completion of NewbornDx prior to confirmation testing	Interval: Hours; single endpoint	Modified Intention-to-Treat Dataset
Time to aresult by rWGS*	Among all babies tested, comparison of date/time of sample collection to date/time of completion of rWGS prior to confirmation testing	Interval: Hours; single endpoint	Modified Intention-to-Treat Dataset
Time from enrollment to a positive result	Among those who have a confirmed positive results at one year CGA by NewbornDx, rWGS or another genetic test,	Interval: Hours; single endpoint	Time to Molecular

Primary Endpoints	Definition	Scale and measure	Populations for which the analysis will be conducted
detected by NewbornDx*	comparison of date/time of informed consent to date/time of completion of NewbornDx prior to confirmation testing		Diagnosis Dataset
Time from enrollment to a positive result detected by rWGS*	Among those who have a confirmed positive results at one year CGA by NewbornDx, rWGS, or another genetic test, comparison of date/time of informed consent to date/time of completion of rWGS prior to confirmation testing	Interval: Hours; single endpoint	Time to Molecular Diagnosis Dataset
Clinical utility of genomic sequencing	If clinical action was taken due to return of results Time from result to action taken Types of clinical actions taken (i.e. surgery, medication) Clinician opinion of utility	Binary: Yes/No Interval Categorical Ordinal	Modified Intention-to-Treat Analysis Dataset
If trio testing was required to obtain a diagnosis for rWGS	If Trios were done If Trios increased diagnostic yield	Binary: Yes/No; single endpoint Binary: Yes/No; single endpoint	Modified Intention-to-Treat Analysis Dataset
If enrollees did not receive a diagnosis	No confirmed diagnosis of a genetic disorder identified by any of the genomic sequencing tests	Binary: Yes/No; single endpoint	Modified Intention-to-Treat Analysis Dataset

*The infants who met the criteria for urgent testing may be analyzed separately.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Secondary Endpoints
1. One-year cost-effectiveness of NewbornDx and rWGS compared with SOC testing, from a societal perspective
2. Lifetime year cost-effectiveness of NewbornDx and rWGS compared with SOC testing, from a societal perspective

We will conduct an economic evaluation that will compare costs and health outcomes for infants with suspected genetic disorders who undergo: 1) traditional standard of care diagnostic testing, 2) NewbornDx, or 3) rWGS. One-year post-identification outcomes for NewbornDx and rWGS will be estimated from trial data, while outcomes for standard of care will rely on retrospective chart review. We will use simulation modeling to extend our projections beyond 1-year post identification to a lifetime time horizon.

In Trial Data Collection for NewbornDx and rWGS:
This clinical trial provides a unique opportunity to examine observed health outcomes, care utilization, and family resource use for infants being evaluated for a suspected genetic disorder. During the trial, charge and cost data will be collected from the total hospital bill at discharge home from the index hospitalization in which the infant is enrolled in the study. Following discharge, the parent will be contacted every 30-days to collect resource utilization related to medical visits, hospitalizations, diagnostic tests, labs, procedures, and medications administered after discharge home until one year of age corrected for prematurity. Data will also be collected on: 1) mother and father lost work days; and 2) observed health outcomes, including death, diagnoses, and the timing of diagnosis. We will use a quality of life instrument to measure parental quality of life at monthly follow-up and to infer the value parents place on avoiding adverse health conditions. We will also collect parent-reported quality of life of the infant using a visual analog scale. Health economics practice guidelines call for inclusion of such outcomes, particularly in child health intervention evaluations[59,60].

Assessment of SOC testing
As no infant in our trial will only receive SOC testing only (i.e., without NewbornDx and rWGS), we will estimate standard of care testing outcomes (costs, diagnostic findings, health outcomes) by using a retrospective chart review.
In the retrospective chart review, we will collect resource utilization and health care outcome data from 300 infants born 12-18 months prior to the start of the study who had a genetic evaluation, but did not receive NewbornDx and rWGS. We chose infants born in this timeframe to minimize differences between contemporary clinical practice and clinical practice received by our retrospective cohort, hence maximizing the generalizability of our retrospective data to contemporary settings.

Simulation: Extending outcome estimates to a lifetime time horizon

Following data collection, we will use simulation modeling to estimate outcomes (cost, and health outcomes) for all strategies: NewbornDx, rWGS and standard of care diagnostic testing over a 1-year and lifetime horizon. For each strategy, the simulation model will project disorder progression based on estimated health state transition probabilities. By associating each health condition (or "health state") with a "utility weight" (QALYs gained per year in that state) and annual cost, the simulation will track hypothetical infants and sum QALY gains and incurred costs. Repeating this process with a large number of simulated infants will yield estimated population health and cost outcomes. Costs and utility weights will be estimated from trial data, and previously published estimates, making the assumption that these values depend only on the infant's health state and not on the diagnostic testing technology. Thus, we assume that the costs and utility weight estimates apply to infants undergoing any of the three diagnostic testing approaches.

Since NewbornDx and rWGS will identify a large number of disorders, disorders will be grouped based on estimated life expectancy and long-term severity. We will identify "disorder groups" by reviewing available data and literature on the expected outcomes of each identifiable disorder in conjunction with expert clinical judgment. We will also identify health states that appropriately represent the possible outcomes for each "disorder group" following review and synthesis of available evidence with input from our Steering Committee and DSMB.

Our lifetime simulation model will require: 1) classification of health states that describe health outcomes associated with each study strategy (e.g. disorder, disability, death); 2) health state transition probabilities, which represent the likelihood that simulated patients will develop specific disorder and disability outcomes, conditional on their assigned strategy; 3) health state costs, which include both health care associated with a specific health state and other societal costs; and 4) health state utilities, which represent health state preferences for avoiding adverse health outcomes. For the time period extending beyond the 1-year CGA trial duration, we will identify model transition probabilities, health state costs, and health utilities from existing primary datasets and the literature. For many genetic disorders, the literature reports limited long-term follow up data that appropriately account for expected differences in health outcomes due to differences in time to diagnosis. Available data will guide short-term extrapolation beyond our trial timeframe. For long-term time periods for which there are no informative data, we will draw on expert opinion, and conduct appropriate sensitivity analyses to characterize the impact of the attendant uncertainty. Health state costs will include the health care and societal costs (e.g. family costs, special education). We will estimate health care utilization, family time and other resources use for each health state from available data sources and then multiply these utilization estimates by national unit prices. We will also estimate child health state utilities using available data and will include utility values for parental quality of life when possible.

Cost-effectiveness analysis: With simulation findings corresponding to the 1-year CGA trial follow-up period and for extrapolation to a lifetime horizon, we will: 1) calculate

aggregate population costs and health outcomes (QALYs) for each trial strategy; 2) rank the strategies from least to greatest aggregate population health (total QALYs); and 3) calculate incremental QALYs and costs for the second strategy (compared to the first) and for the third strategy (compared to the second). Incremental QALYs are, by our sequencing of these comparisons, non-negative. If corresponding incremental costs are likewise non-negative, an incremental cost-effectiveness ratio (ICER) (incremental costs divided by incremental QALYs) will be calculated in a similar fashion. If incremental costs are negative in either case – that is, if either strategy reduces costs while improving health relative to its comparator – we refer to this strategy as "cost-saving".

Secondary Endpoint	Definition	Scale	Populations for which the analysis will be conducted
Satisfaction of the web-based clinical reference database	Usefulness of information Ease of use of the clinical reference database	Categorical	Medical providers who use the clinical reference database after it is built

In order to qualitatively assess the impact of the intervention, we will collect surveys from medical providers across all sites. This survey will be submitted to the IRB prior to implementation.

9.4.4 SAFETY ANALYSES

All-cause mortality will be evaluated at discharge home from the hospitalization during which enrollment occurs and at one-year CGA. Data will be reviewed to determine if diagnoses are made in a timely fashion and if the diagnoses are reported accurately.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Population description: Gestational age, birth weight, day of life at admission, and length of stay in the hospital will be summarized with means, medians, standard deviations, inter-quartile ranges, and 95% confidence intervals (CI). Prenatal evaluations, complications, and mode of delivery will be summarized with frequencies and percentages. Signs/ symptoms that warranted the testing will be summarized with frequencies and percentages.

9.4.6 PLANNED INTERIM ANALYSES

Interim analyses are not planned.

9.4.7 SUB-GROUP ANALYSES

The primary or secondary endpoints will not be analyzed by demographic sub-groups; we do not expect different results according to sex or race/ethnicity.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

N/A

9.4.9 EXPLORATORY ANALYSES

A review of the conditions detected by rWGS but not NewbornDx will be conducted and this review may potentially improve the NewbornDx panel.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent materials for this protocol include and the Informed Consent Form and a Secondary Findings Parent Handout, both of which will be IRB-approved prior to use. The ICF will describe in detail study procedures and risks and will include check boxes to elect to receive secondary findings for the infant and each biological parent. The same informed consent forms and when applicable, assent forms, will be used at all sites. The ICF will be translated into Spanish. Short-forms will be used for all languages other than English or Spanish.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Documentation of Consent
Written documentation of informed consent is required prior to enrolling in the study or conduct of any study procedures with the exception of IRB-approved recruitment efforts and the unusual circumstance described in #7 of the telephone consent procedures. Written documentation of consent is required for the participation by the infant and each biological parent providing a blood sample.

Consent when the Parent/Guardian of the Infant or a Biological Parent is ≥ 18 Years
Participation of the infant requires written documentation of consent by one parent/ guardian. Participation of a biological parent requires written documentation of consent by that biological parent.

Consent when the Parent/Guardian of the Infant is < 18 Years Old

State	Who provides consent for the Infant's participation?	Must a minor parent provide assent for the infant's participation?
California	Minor parent	N/A
Massachusetts	Minor parent	N/A
New York	Minor parent	N/A
North Carolina	One guardian of the minor parent	No
Ohio	Minor parent	N/A
Pennsylvania	Minor parent	N/A

Consent when a Biological Parent is < 18 Years Old

State	Who provides consent for participation by the minor parent?	Must a minor parent provide assent for his or her own participation?
California	One guardian of the minor parent	Yes
Massachusetts	Minor parent	N/A
New York	Minor parent	N/A
North Carolina	One guardian of the minor parent	Yes, assent signature line on ICF
Ohio	One guardian of the minor parent	Yes, assent signature line on ICF
Pennsylvania	One guardian of the minor parent	Yes, assent signature line on ICF

Assent for a minor parent's participation is required by 4 sites. North Carolina Children's Hospital, Children's Hospital of Pittsburgh and Cincinnati Children's Hospital Medical Center will use an additional assent signature line on the ICF. A separate assent form for participation by a minor parent will only be used by Rady Children's Hospital - San Diego and submitted to the central IRB at the time of their site IRB application.

By signing the informed consent form, the infant's parent/guardian agrees that the infant will complete all evaluations required by the study, unless the infant's parent/guardian withdraws the infant voluntarily or the infant is withdrawn from the trial for any reason.

The original signed ICF and if applicable, assent documents, will be put in the infant's research file. A copy of the signed informed consent/assent documents will be:

- provided to the parent/guardian(s) and biological parent(s) as applicable
- put into the infant's medical record and if applicable, each parent's medical record

An entry into the infant's medical record will indicate informed consent was obtained (refer to the Documentation of Informed Consent template).

Process of Informed Consent

Informed consent is a process initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation.

1. Research staff at the enrolling site will communicate with the clinical team prior to approaching a parent/guardian. The clinical genetics team will be consulted as dictated by SOC at each site. The clinicians may be asked to speak with the parent/guardian(s) prior to their approach by the research team depending on family and clinical circumstances.

2. The parent/guardian(s) of the infant and as applicable, the biological parents of the infant, will be approached directly for consent. If the parent/guardian(s) are not directly available, they may be contacted via telephone in extenuating circumstances (see telephone consent below).
3. Research staff will provide a comprehensive summary of the study including contact information for the research team, and if requested, consent/assent documents.
4. The site-PI, co-investigator or a study coordinator who is experienced in genetic studies will obtain consent. This person will verbally explain the research study and answer any questions. The information available on each genetic sequencing platform including primary findings, secondary findings and the risk of finding out about mistaken paternity or suspected incest will be described (refer to section 2.3 Risks/Benefits). The following will be emphasized: participation is voluntary; the infant may be withdrawn from the study at any time without prejudice, and the decision not to participate will in no way adversely affect the quality of their infant's medical care.
5. The parent/guardian(s) and biological parents will have the opportunity to review the written consent form and ask questions prior to signing. Parent/guardian(s) will be given as much time to consider the study and read the consent documents as necessary.

<u>Non-English Speakers</u>

1. For non-English speaking families, a fully translated standard consent or an oral presentation accompanied by a short form may be used to obtain informed consent. The fully translated consent and the short form will be approved by the central IRB prior to its use.
2. A Spanish translation of the informed consent form will be available; a short form will be used for all other languages. If a short form is not available for the family's preferred language, consent will be obtained through a certified interpreter and documented on the English consent form. Short forms approved by the Johns Hopkins IRB can be found here: https://www.hopkinsmedicine.org/institutional_review_board/forms/short_form_translation.html
3. An interpreter from the hospital will be called to assist with the consent process if needed. When an interpreter is not available in-person, a professional phone interpreter service may be used. Family members should not serve as an interpreter due to the complex nature of genetic testing.
4. Interpreters may also serve as a witness to the short form consent process; their signature as an interpreter (or an interpreter reference ID number from a professional telephone interpreter service) will serve as a witness signature. The non-English speaking participant signs the short form consent in their language. The person obtaining consent signs the English version full consent. The interpreter/witness understands both languages, so signs both.
5. The parent/guardian(s) and biological parent(s) who sign the consent documents will be given a copy of these documents.

Telephone consent

It is the intention of the study team to obtain written consent from the parent/guardian for the infant and each biological parent if providing a sample whenever possible. It is anticipated that in certain circumstances, it may be necessary for the consent process to be conducted via telephone with written documentation of consent to follow. Examples of when this might be needed include: when the biological mother remains at the birthing hospital after her infant is transferred to the enrolling site and a parent/guardian or biological parent cannot physically visit because the family lives too far away. If the consent process is not able to be conducted in-person, telephone consent may be used with written documentation of consent to follow.

The PI will ensure the procedures for securing telephone consent are followed:

1. The absent party will be contacted by phone. The research staff will explain the entire research study and the process required for consent by telephone. An email address or fax number will be obtained in order to forward the consent document(s). A time will be scheduled for a full informed consent when both parties will be able to have the consent physically in front of them to sign. For non-English speaking persons, refer to procedures for Non-English speaking persons above.
2. The consent document(s) will be sent to the absent party. The absent party will be advised that they are welcome to read this document, but they will be asked not to sign the document until the time of the scheduled consent.
3. At the time of the scheduled consent, the research staff will have a witness present to confirm that the telephone consent was conducted. All study information will be discussed, and any questions answered.
4. Following the explanation of the study, the absent party will be asked to sign and date the document. The research staff and witness will also sign and date their copy.
5. The absent party will be asked to return the signed document to the research team, either electronically or if necessary, by mail.
6. Copies of both signed documents will be made and returned to the absent party.
7. Written documentation of consent will be obtained prior to any study activities with one exception. In the circumstance where testing is urgent (clinical situation is life-threatening) and written documentation of consent is not readily feasible (i.e. no access to a fax machine or scanner), verbal consent as documented by the research staff will be sufficient to obtain, ship and process blood samples. However, written documentation of consent by the parent/guardian must be documented prior to the return of any results.
8. If necessary, the research staff will attempt to arrange for a blood collection kit to be delivered to the absent party. In the case where the biological mother is at another hospital and wishes to provide a blood sample, we will work with the birthing hospital to collect the sample and return the consent documentation.
9. The consent will be documented per hospital and IRB policies.

New Information

Any new information that may affect willingness to continue in the study will be communicated by the investigator to the parent/guardian(s) or biological parent(s) in

accordance with IRB requirements. The informed consent document will be updated and re-consent will be obtained if necessary.

Waiver of Consent
Waiver of consent is requested for the clinician survey. After the genomic sequencing result is returned for an infant, the physician of record for the infant will complete a survey about changes in care from study results and perceived utility of testing.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is reasonable cause. The trial will be stopped for a safety review by the DSMB if at any point the act of conducting the trial is deemed detrimental or unsafe to infants. Reasons for stopping the trial may include the reporting of inaccurate test results due to either problems with the technology or the medical information interface. The trial may also be stopped if a data breech has occurred that puts an infant's personal genomic data at risk. Formal stopping rules will be developed with the DSMB prior to the trial beginning.

If the study is prematurely terminated or suspended, written notification documenting the reason for study suspension or termination will be provided by the study-wide Principal Investigator (PI) to site investigators, NCATS, the DSMB and the IRB. The site investigators will promptly inform the parent/guardian(s) of enrolled infants who have not yet completed the study and inform them of any changes to study procedures.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to infants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the DSMB, NCATS and the IRB.

10.1.3 CONFIDENTIALITY AND PRIVACY

Confidentiality will be maintained by the participating investigators, their staff, and the sponsor(s). This confidentiality covers the clinical information relating to participating infants, testing of biological samples and genetic tests.

All study related material will be stored securely by the site. Each enrolled infant will be assigned a unique study identification number which does not include any identifiers. The information entered into the password-protected REDCap System and the documents used for remote monitoring will be identified by the Infant Study ID number. Each site will keep the link between the Infant Study ID number and infant identifiers for

all subjects enrolled at their site in a secure manner (password-protected spreadsheet or hand-written log securely locked).

The ICF will clearly indicate that the labeling of specimens, medical information and reports to/from the laboratories will contain identifying information. Confirmed genomic testing results will be returned from the laboratory to the site and the infant's EMR and will contain patient identifiers. All genomic sequencing data will be electronically stored in password-protected databases at the laboratory that conducted the test.

Paper-based study files, including the original ICF/assent documents and contact information related to the infant will be stored in a secure office of the research staff for internal use during the study. A copy of the signed ICF will be placed in the infant's medical record. At the end of the study, all records will continue to be kept in a secure location (i.e. a secure storage facility) for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study research data about the infant, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Tufts Medical Center Data Coordinating Center. The REDCap and study management systems used by clinical sites and by Tufts Medical Center research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at Tufts Medical Center.

Per Section 2012 of the 21st Century Cures Act as implemented in the 2017 NIH Certificates of Confidentiality Policy, this study is automatically issued a COC. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants. The fact that a COC may not apply in all circumstances is disclosed in the ICF.

Clinical information will not be released without written permission of the infant's parent/guardian, except as necessary for monitoring by the research staff for this study, IRB, DSMB, NIH, or OHRP. Senior project leadership or other authorized representatives may inspect all documents and records required to be maintained by the PIs. This documentation includes, but is not limited to medical records (office, clinic, or hospital) for the infants in this study. Clinical study sites will permit access to such records.

<u>Protection against a data security breach</u>

- Paper-based study files, including original signed ICFs, will be stored in a locked office of the study team members or a secure storage facility. A copy of the

signed ICF will be placed in the subject's medical record (paper or scanned in to the EMR as applicable). All genomic sequencing data will be electronically stored accessible only to research personnel.

- The RCIGM ordering portal to which each infant's medical record is uploaded is HIPAA-compliant and requires a login and password

- Any PHI distributed over email (return of results) must be encrypted and distributed via secure institutional email systems. Results will be returned to the electronic medical record consistent with each site's HIPAA-compliant institutional policies.

- Electronic study files containing PHI (i.e. a screening log) at each site must be kept secure (i.e. password-protected file in a restricted access folder maintained on a secure internal institutional server)

- The REDCap system is password protected; limited staff at each site will be granted password login ID. All personnel must provide an active organizational email address before being issued an ID. The REDCap system requires complex passwords, and the frequency of forced password changes is every 90 days. Enrolling sites will only have access to data entered into the REDCap system for infants enrolled at their own site with the exception of the study-wide PIs, DCC and monitoring personnel who will have access to all sites' data. Enrolling sites will not have access to export study data.

- The data center in which the REDCap servers are housed has strict access control; only authorized core personnel may access the facility unescorted. Only authorized users are allowed to connect to the network and the security of the network is actively monitored. Power and environmental controls have several layers of backups. The institution actively logs and monitors all communication to the server (multiple firewall layers prevent direct external communication to the server) and is alerted to any unusual activity. If warranted, the institution will immediately as well as automatically ban offending IP addresses at the perimeter before they reach the server. The application itself also rejects and bans IP addresses of anything it considers abnormal access. REDCap Server(s) are regularly backed up. All transactions are securely delivered to the application using SSL (SHA-1 with RSA Encryption; 2048-bits). It is then transmitted internally (behind the firewall) to the database server. All transactions are logged at the server layer (http logging), application layer (REDCap logs activity to a database table), and the database layer.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Blood samples/DNA

Blood samples/DNA will be kept by the laboratories for the duration of the study period (up to 5 years) for the purpose of further testing of the infant. The specimens will then be destroyed at the conclusion of the study.

Raw Sequencing Data

The raw sequencing data generated by this research will be kept by Athena (Quest) Diagnostics and RCIGM for at least the length of the 5-year study period. The laboratories may destroy raw sequencing data after the conclusion of the study but should not do so without permission from the study wide PIs. Prior to destruction of any raw sequencing data, genomic sequencing data may be:

1. Used to re-analyze VUS; in the event an infant's diagnosis changes, we will attempt to contact the parents
2. De-identified and transferred to a third party for use in a future study to further understand neonatal and childhood diseases

Sharing of Genomic Research Data

This prospective, multi-center clinical trial will generate genomic sequencing data that is classified as 'Level 4'Data by the Genomic Data Sharing (GDS) Policy of the National Institutes of Health. Sequencing data that relates genomic data to phenotype or other biological states will be generated. As such, all human genomic data will be released in accordance to the NIH GDS Policy which establishes the timelines for submission and subsequent release of data for access by secondary investigators. Specifically, data (results of testing) will be provided to infants and their caregivers when complete and validated (with the exception of provisional results which are given prior to confirmation). In addition, data, including genome sequences (fastq files), variants (vcf files), and associated HIPAA compliant clinical metadata will be deposited in the Longitudinal Pediatric Data Resource (LPDR; https://www.nbstrn.org/research-tools/longitudinal-pediatric-data-resource). The LPDR is being developed by the Newborn Screening Translational Research Network (NBSTRN). The LPDR is a secure informatics system designed to enable enhanced data collection, sharing, management and analysis for conditions identified as part of newborn screening or for conditions that may benefit from newborn screening, including NICU genomic sequencing. The NBSTRN is funded by the NICHD (contract #HHSN275201300011C). The LPDR is currently being used to deposit data from infants in the NICU and PICU who have already had rWGS performed related to the NICHD/NHGRINSIGHT program (PI Dr. Kingsmore). Much of the infrastructure for those datasets will serve as a template for the dissemination of data from this proposal. The LPDR, in turn, will deposit data in the Controlled Access section of the NCBI dbGAP. Variants with ACMG recommended pathogenicity assessments will be deposited in ClinVar. Novel disorder gene assertions will be deposited in ClinGen (*https://clinicalgenome.org/*). We anticipate annual data submissions supplemented by specific dataset deposits as manuscripts arising from this work are submitted for publication.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Co-Principal Investigators:

Jonathan M. Davis, MD
Tufts Medical Center
800 Washington Street, Box 44, Boston, MA 02111
Tel: 617-636-5322
jdavis@tuftsmedicalcenter.org

Jill Maron, MD, MPH
Tufts Medical Center
800 Washington Street, Box 44, Boston, MA 02111
Pager: 617-647-2971
jmaron@tuftsmedicalcenter.org

10.1.6 SAFETY OVERSIGHT

DSMB
Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB). The DSMB is composed of individuals with the appropriate expertise, including an ethicist, geneticist, neonatologist and statistician:

Tamsin Knox, MD, MPH (CHAIR)
Associate Director, Regulatory Group, Tufts Clinical and Translational Science Institute
Tufts University School of Medicine
150 Harrison Avenue, Jaharis 2, Boston, MA 02111
Tel: 617.636.3558
tamsin.knox@tufts.edu

Jonathan M. Fanaroff MD, JD
Professor of Pediatrics
Case Western Reserve University School of Medicine
Director, Rainbow Center for Pediatric Ethics
Co-Medical Director, Neonatal Intensive Care Unit
Rainbow Babies & Children's Hospital
Cleveland, Oh 44106

Gina M. Geis, MD, MS
Attending Neonatologist
Associate Director, Neonatal-Perinatal Medicine Fellowship Program
Associate Professor of Pediatrics
Associate Professor in the Center for Bioethics, Education and Research
Albany Medical Center
Department of Pediatrics, Division of Neonatology
Albany, NY 12208

Stephanie Sacharow, MD, FACMG
Assistant Professor, Harvard Medical School
Medical Genetics, Medical Biochemical Genetics
Boston Children's Hospital
Division of Genetics and Genomics

Hong Chang, PhD
Statistician and Senior Project Manager, Biostatistics, Epidemiology, and Research Design (BERD) Center
Assistant Professor
Tufts University School of Medicine
Boston, MA

Members of the DSMB are independent from the study conduct and free of conflict of interest. The DSMB will meet at least annually to assess safety data or more often if needed. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element the DSMB needs to assess will be clearly defined. The DSMB will provide its input to NCATS.

Steering Committee
The Steering Committee (Drs. Davis, Diacovo, Gelb, Ginns, Kingsmore, Maron, Poindexter, Powell and Vockley) with significant expertise in neonatal clinical trials and genomic medicine/testing will oversee the conduct of the trial. The Steering Committee will have conference calls every three months (or more often if needed) to review any problems, issues, and operations for the study.

10.1.7 CLINICAL MONITORING

Clinical site monitoring will be conducted remotely by Tufts Medical Center research staff to ensure that 1) the rights and well-being of trial participants are protected; 2) the reported trial data are accurate, complete, and verifiable; and 3) the conduct of the trial is in compliance with the currently approved protocol/ amendment(s) and with applicable requirement(s) as outlined in the DSMP.

- Data monitoring will be conducted remotely (enrolling sites will upload de-identified medical records and source documents to the REDCap system for reference by monitoring personnel)

- Data monitoring will consist of examination of data and processes related to informed consent, subject eligibility, the accuracy of study primary endpoints, and safety/adverse events as outlined in the DSMP

- Although monitoring will occur throughout the study, the frequency of monitoring will vary for each specific process or data point as outlined in the DSMP

- The study-wide Co-PIs will be provided copies of monitoring reports quarterly
- Monitoring will include, but are not limited to, review of essential documents, accountability records, CRFs, ICFs, medical and laboratory reports, and protocol compliance
- Each site principal investigator will provide direct access to study-related documents for monitoring and auditing by the data coordinating center or its representative, and inspection by local and regulatory authorities
- If a significant number of issues at a site are identified, additional training, increased remote monitoring or in-person monitoring may occur

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

The site PI will provide direct access to all trial-related sites, source data/documents, case report forms, and reports for the purpose of monitoring and auditing by Tufts Medical Center, and inspection by local and regulatory authorities. The site PI will also ensure that all study personnel are appropriately trained and the applicable documentation is maintained on site.

Tufts Medical Center research staff will verify that the clinical trial is conducted, and the data generated, documented (recorded), and reported in compliance with the protocol and 45 CFR Part 46 as outlined in the DSMP.

The DCC will implement quality control procedures beginning with the data entry system and generate data quality control checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Any missing data or data anomalies will be communicated to the site(s) for prompt clarification and resolution.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of site staff under the supervision of the site PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. Adverse events must be graded, assessed for severity and causality, and reviewed by the site principal investigator or designee.
The DCC for this study will be responsible for data management, quality review, analysis, and reporting of the study data.

Data for each participant will be captured on eCRFs. Study personnel at each site will enter clinical data from the medical record or source document directly into REDCap. The data system includes password protection and internal quality checks, such as automatic range checks, tracking of missing data, and the ability to create and track manual queries. The medical record will serve as the source documents in most cases. However, some data may need to be captured separately on a source document (for

example, date and time of sample collection). All source documents should be completed to ensure accurate interpretation of data. When making a change or correction, the original entry should be crossed out with a single line, and the change should be initialed and dated. Avoid the use of erasers, overwriting, or correction fluid/tape on the original. Data reported in the eCRF should be consistent with the source documents or the discrepancies should be documented in a note-to-file.

10.1.9.2 STUDY RECORDS RETENTION

Records and documents pertaining to the conduct of this study, including source documents, consent forms, and laboratory results must be retained by the investigator for at least 7 years after the end of the study or longer as applicable to local/state/federal regulations. No records will be destroyed without the written consent of NIH. It is the responsibility of the overall study PIs to inform the site investigators when these documents no longer need to be retained.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any non-compliance with the clinical trial protocol or manual of procedures requirements. The non-compliance may be on the part of the participant, the investigator, or the study site staff. It is the responsibility of the site to identify protocol deviations and to promptly develop and implement corrective actions. All deviations from the protocol must be reported in REDCap under the protocol deviation eCRF within 5 working days of identification. A completed and investigator-signed copy of the protocol deviation form must be maintained in the file of essential documents. Protocol deviations must be submitted to the IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to the central IRB requirements.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

- The National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research, requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. The policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.
- This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers after the completion of the primary endpoint by contacting the Co-PIs Jonathan Davis or Jill Maron.

- This study will also comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data. Refer to section 10.1.4 of this protocol for further information.

In addition, the following groups may share the data to improve new treatments or other clinical trials (this language will be included in the ICF):

- Health authorities throughout the world (e.g., Food and Drug Administration, European Medicines Agency, NIH, etc.)
- Institutional Review Boards
- Other groups such as academic, government, or industry researchers; public-private partnerships; and/or external research collaborations. These entities will have oversight committees that will supervise the ethical use of the data

The Steering Committee will be responsible for developing publication procedures and resolving authorship issues.

If the parent/guardian would like to enroll the infant or him or herself in another research study that will utilize sequencing data, the enrolling site/labs may give the raw sequencing data from the GEMINI study directly to the other study upon receipt of a HIPAA authorization form signed by the infant's parent/guardian.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence is critical. Therefore, any conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. Site investigators/study team members must adhere to their local requirements for disclosure of conflicts of interest and the local site reviews and manages any reported conflicts of interests. The local site must provide a copy of each research team members' reported conflict of interests to 1) the JHU IRB and 2) the study-wide co-PIs. The JHU IRB will receive any management plans from relying sites and review them as part of oversight/review responsibilities but does not perform the initial review instead of the local site nor manage reported interests.

11 ABBREVIATIONS

ACMG	American College of Medical Genetics and Genomics
AE	Adverse Event
ASHG	American Society of Human Genetics
BW	Birth Weight
BWA	Burrows-Wheeler Aligner
CFR	Code of Federal Regulations
CGA	Corrected Gestational Age
CLIA	Clinical Laboratory Improvement Amendments
CNVs	Copy Number Variants
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DBS	Dried Blood Spot
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DRE	Disease-Related Event
DRG	Diagnosis-Related Group
DSMB	Data Safety Monitoring Board
DSMP	Data Safety and Monitoring Plan
EC	Ethics Committee
eCRF	Electronic Case Report Forms
EMA	European Medicines Agency
EMR	Electronic Medical Record
FDA	Food and Drug Administration
GA	Gestational Age
GATK	Genome Analysis Toolkit
GCP	Good Clinical Practice
GDS	Genomic Data Sharing
GINA	Genetic Information Nondiscrimination Act
GLP	Good Laboratory Practices

GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
HPO	Human Phenotype Ontology
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IGV	Integrated Genome Viewer
INC	International Neonatal Consortium
IRB	Institutional Review Board
LAR	Legally Authorized Representative
MOP	Manual of Procedures
N	Number (typically refers to participants)
NBS	Newborn Screening
NCATS	National Center for Advancing Translational Sciences
NICU	Neonatal Intensive Care Unit
NIH	National Institutes of Health
NPV	Negative Predictive Value
OHRP	Office for Human Research Protections
OMIM	Online Mendelian Inheritance in Man
PCR	Polymerase Chain Reaction
PHI	Protected Health Information
PI	Principal Investigator
PMA	Postmenstrual age (gestational age plus postnatal age)
PNA	Postnatal age
PPV	Positive Predictive Value
QA	Quality Assurance
QALY	Quality-Adjusted-Life Year
QC	Quality Control
RCIGM	Rady Children's Institute for Genomic Medicine
rWES	Rapid Whole Exome Sequencing
rWGS	Rapid Whole Genome Sequencing

SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SNP	Single nucleotide polymorphism
SOA	Schedule of Activities
SOC	Standard of Care
SOC	System Organ Class
SOP	Standard Operating Procedure
STR	Short Tandem Repeat
Sub-I	Sub Investigator
SVs	Structural Variants
NewbornDx	NewbornDx Sequencing Evaluation
UP	Unanticipated Problem
US	United States
WES	Whole Exome Sequencing
WGS	Whole Genome Sequencing

12 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale.

Version & Date	Description of Change	Brief Rationale
2.0, 05DEC2019	The term incidental has been added to sections that refer to secondary findings.	The RCIGM laboratory report will use the term incidental findings, instead of secondary findings, to reflect that the lab does not conduct a *targeted* search of variants not related to the infant's phenotype.
2.0, 05DEC2019	When samples for the infant and parent(s) are received at the same time, RCIGM will routinely generate infant and parent sequences simultaneously and the data all analyzed together as a duo/trio as applicable.	To increase diagnostic yield and/or decrease the time to result for the subject; NewbornDx is run as a trio when parent samples are available.
2.0, 05DEC2019	A retrospective analysis of infant-only data among infants who were initially analyzed as a duo or trio will be conducted.	To provide data about how often a trio is needed to obtain the infant's result.
2.0, 05DEC2019	The laboratory testing processes for subjects from Mount Sinai has been revised to reflect the new status of NewbornDx as conditionally approved by New York State and the ramifications of this approval on existing processes.	To match the already IRB approved processes included in the Mount Sinai Local Context Questionnaire as a result of the conditional approval of NewbornDx by New York State.
2.0, 05DEC2019	The adjudication of discordant results procedures has been revised and includes the view by the 1) clinician directly caring for the subject and 2) a subset of the steering committee of the result that aligns best with the infant when discordance is due to variant classification.	To better structure the process and to obtain the view of the clinician caring for the infant and an impartial sub-committee.

2.0, 05DEC2019	The exclusion criteria “Infants who are not expected to receive medical care in the US healthcare system from time of discharge home until 1 year CGA” has been added.	To ensure comparable follow-up economic data can be used in the health economic analysis.
2.0, 05DEC2019	Text to clarify that enrollment in this study is not intended to replace targeted available genetic testing for obvious suspected disorders have been added.	To clarify that enrollment should not be prohibited nor delayed for return of clinical genetic test results unless the suspected disorder is of an obvious nature and therefore rWGS is not needed.
2.0, 05DEC2019	Terminology within the primary endpoints definitions has been updated.	To use consistent terminology across endpoints and definitions.
2.0, 05DEC2019	The option to obtain 1ml of blood in an EDTA tube in lieu of the blood spot card when the 3-hour drying time for the blood spot card pushes the shipment of samples to the next day and the case is urgent.	To allow flexibility in sample collection vehicle in urgent cases when waiting for the drying time of a dried blood spot card would adversely affect time to potential life-saving treatment.
2.0, 05DEC2019	Details about the following have been deleted and moved to the MOP and/or eCRF instructions: Identification number definitions; description of eCRF; blood sample collection procedures; Athena specimen ID creation example; RCIGM portal instructions; shipping instructions and shipping addresses; contact information; source document types.	To streamline repetitive information that is more appropriate for the MOP.
2.0, 05DEC2019	A licensed genetic counselor may return results without an attending physician present if clinical practice is for the licensed genetic counselors to do so outside of this study.	To align with the existing clinical workflow at a site in a state for whom genetic counseling licensing allows for return of results.
2.0, 05DEC2019	Procedures for when, how and by whom results are returned	To ensure consistent procedures across sites.

	after an infant is discharged home have been added.	
2.0, 05DEC2019	The site should wait until results from both NewbornDx and rWGS are returned before speaking with the parent/guardian about the results if waiting would not harm the infant (i.e. the result is not provisional or the infant does not require urgent testing).	To clarify the intended timing of communication with the infant's parent/guardian(s).
2.0, 05DEC2019	The timeframe in which to collect the Clinical Utility Survey has been expanded to include at discharge home or for infants who discharge home prior to the return of results, within one month after the return of results.	To accommodate varying scenarios of when results are returned/acted upon.
2.0, 05DEC2019	In cases in which the infant is discharged home prior to the return of results, the Clinical Utility Survey should be completed by the clinical geneticist, most relevant subspecialist or by the site PI after speaking with the pediatrician.	To account for different physicians who will follow the subject and act upon results as an outpatient.
2.0, 05DEC2019	Physician CPT codes have been added to the types of data captured.	To better describe the types of billing data collected.
2.0, 05DEC2019	A parent may only opt-in to receive secondary findings if the parent/guardian opted-in for infant secondary findings. Inclusion of a table detailing the results returned for the infant and parent based on opt-in status, disorder onset, if the result is included on the ACMG59 or medically actionable.	To clarify under what circumstances a parent may opt-in for additional results and the possible results returned for the infant and parent.
2.0, 05DEC2019	Removed clinical geneticist as a required input to eligibility discussion. The study team will discuss eligibility of the infant	Depending on the site and condition, genetics is not always consulted per SOC.

	with the attending physician of record and if in agreement, the family will be approached for the study.	
2.0, 05DEC2019	The screening data captured in REDCap has been changed to replace maternal education and income level data with 5-digit zip code and primary symptoms with primary system involvement related to patient nomination.	The 5-digit zip code will be used in the health economic analysis. The change from clinical symptoms to primary system(s) involvement related to patient nomination of infants who have not provided consent is to reduce the risk of identification given the addition of zip code.
2.0, 05DEC2019	Revised consent via telephone procedures. If the infant's clinical status is life threatening and in-person consent or written documentation of consent via telephone is not feasible, verbal consent by telephone as documented by the research staff is sufficient to obtain, ship and process blood samples; however documentation of written consent must be obtained prior to return of any results.	To enable the timely conduct of research procedures for infants whose clinical status is life threatening, whose parent/guardian verbally consents to participate in the study but does not have immediate access to a fax/scanner to return the signed ICF, and the potential delay in written consent may prevent life-saving treatment.

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14 APPENDICES

- Informed Consent Form
- Secondary Findings Parent Handout
- Follow-up Contact Information Form
- Resource Utilization Follow-up Email and Questionnaire
- SF-12 Your Health and Well Being
- Child Visual Analog Scale instrument
- Authorization to Release Protected Health Information
- Clinician Assessment of Clinical Utility