Session 3 – Molecular Advances

Monday, Oct. 27 – 3:30pm-5:00pm

Moderators – Suzanne Cordovado, PhD, Centers for Diseases Control and Prevention and Michelle Caggana, ScD, New York State Department of Health

Evaluation of Stored Newborn Screening Specimens from Children Diagnosed with Conditions that May be Identifiable in the Newborn Period by Molecular Testing for Measures of T and B cell Development

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Abstract

Background: The Massachusetts Newborn Screening Program has offered statewide newborn screening for the early detection of severe combined immunodeficiency (SCID NBS) since February 2009. SCID NBS makes use of a DNA-based assay that counts the numbers of TREC, a marker of T cell development (low numbers of TREC being a hallmark of SCID). A DNA-based assay that counts the numbers of KREC, a marker of B cell development, has been described and may be multiplexed with the TREC assay. Use of a TREC/KREC assay offers the potential to expand the number of treatable conditions screened or to provide insights about long term outcomes among infants showing lymphopenia, not SCID.

Objective: To define the TREC and KREC profiles in newborn screening specimens retrieved from older children who now carry a diagnosis of particular primary immunodeficiencies (PID) or other conditions of immune dysfunction.

Hypothesis: Neonates who later present with PID or other conditions of immune dysfunction are likely to have out of range or low-normal TREC or KREC profiles and lymphopenia in the first days and weeks of life.

Method: Patients known to clinicians in the MA SCID NBS Working Group who have particular diagnoses expected to be associated with low levels of TREC or KREC (based on published data, experience with TREC NBS, and on theoretical grounds).were invited to consent to retrieval of their stored newborn screening specimens for testing by TREC/KREC assay. Specimens and anonymized controls were tested using TREC/KREC assays developed in the New England Newborn Screening Program. Condition-specific profiles were determined.

Results: As expected, newborn specimens from infants later diagnosed with XLA show undetectable KREC and normal TREC. Those from one SCID MIA and one ATA have both abnormal TREC and abnormal KREC. Specimens attributed to other non-SCID diagnoses appear to have normal KREC.

Conclusion: Use of a TREC/KREC assay in the primary tier has the potential to identify XLA in addition to SCID. More data are needed.

Summary

More than 400,000 infants have been screened for Severe Combined Immunodeficiency (SCID) since Massachusetts began offering SCID newborn screening in 2009. Four infants with classical SCID, one infant with leaky SCID, 24 infants with DiGeorge (including one with complete DiGeorge), 8 infants diagnosed with other syndromes and another 16 infants with secondary T-cell lymphopenia were identified. In addition, another 24 infants with idiopathic Tcell lymphopenia were identified. We wondered whether infants identified by the screen as having T cell lymphopenia and not SCID might present later with a different primary immunodeficiency or other conditions known to be deficient in T or B cells.

The DNA-based assay used for SCID newborn screening counts the numbers of TREC, a marker of T cell development (low numbers of TREC being a hallmark of SCID). We modified the TREC assay such that it could also count the numbers of KREC, a marker of B cell development. We applied our TREC/KREC assay to define the TREC and KREC profiles in newborn screening specimens retrieved from older children who now carry a diagnosis of particular primary immunodeficiencies (PID) or other conditions of immune dysfuntion.

Method: Patients known to clinicians in the MA SCID NBS Working Group who have particular diagnoses expected to be associated with low levels of TREC or KREC (based on published data, experience with TREC NBS, and on theoretical grounds) were invited to consent to retrieval of their stored newborn screening specimens for testing by TREC/KREC assay. Specimens and anonymized controls were tested using TREC/KREC assays developed in the New England Newborn Screening Program. Condition-specific profiles were determined.

Results: The TREC/KREC assay was validated for this research using a set of newborn screening specimens from infants previously diagnosed with SCID and for whom a T cell/ B cell profile was available. All 8 SCID patients showed undetectable TREC. Two patients diagnosed with ADA SCID also showed undetectable KREC and one patient with PNP SCID showed extremely low KREC. In contrast, 4 patients with IL7RA, IL2RG or likely CD3D SCID all showed copy numbers of KREC well above our working cutoff of 65 copies per microliter of blood.

Every newborn screening specimen from infants later diagnosed with: acute lymphocytic leukemia (ALL; 29 patients, 31 specimens), ALL and trisomy 21 (4 patients 7 specimens), congenital CMV (1 patient, 4 specimens) DiGeorge (8 patients, 10 specimens), trisomy 21 (28 patients, 33 specimens) and Wiskott-Aldrich syndrome (2 patients, 3 specimens) showed KREC levels well above our working cutoff and comparable to their controls that had been matched for age, NICU status and storage conditions. One patient diagnosed with Ataxia Telangectasia showed undetectable TREC and extremely low KREC. Of interest, one patient diagnosed with SCID MIA showed undetectable TREC and KREC on the initial specimen and increasing levels of KREC only on later specimens. Two patients later diagnosed with XLA showed normal TREC and undetectable KREC.
**Conclusion:** Use of a TREC/KREC assay in the primary tier has the potential to identify XLA in addition to SCID. More data are needed to determine a population-based rate of specimens with abnormal KREC results and whether that would be feasible for routine newborn screening. Use of a TREC/KREC assay offers the potential to expand the number of treatable conditions screened or to provide insights about long term outcomes among infants showing lymphopenia, not SCID. Neonates who later present with particular PID are likely to have out of range or low-normal TREC or KREC profiles and lymphopenia in the first days and weeks of life.

**Determination of TREC Copy Numbers from Dried Blood Spots Using Digital PCR**

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**Abstract**

T-cell receptor excision circles (TRECs) are used as the target analyte for identification of infants at risk for severe combined immunodeficiency (SCID). Because SCID is characterized by low or absent T-cells, infants with a positive TREC screen will have few or no T-cells which makes accurate quantification of TRECs difficult. Further, due to different approaches in testing methodology as well as variations in equipment and reagents used for testing, standardization of TREC quantification across laboratories has been difficult. Nearly every newborn screening laboratory performing SCID screening utilizes a different action threshold based on TREC copy number or Cycle threshold (Ct). We attempted to determine absolute TREC copy number using a digital PCR approach which relies upon Poisson statistics to calculate target copy numbers without the need for a standard curve. We prepared and analyzed TREC plasmid samples, laboratory-derived dried blood spot calibrators, and newborn dried blood spot samples. Analysis of known 40 and 10 copy TREC plasmid standards by digital PCR yielded 41.61 and 10.53 copies. Further, comparison of digital data to in situ TREC assay data from lab-derived calibrators returned comparable results (60.59 copies vs 70.88 copies and 11.37 copies vs 6.18 copies). Newborn DBS samples were selected based on in situ TREC assay results and spanned Ct values from approximately 28.50 to 32.50, in 1.0 Ct increments. Digital PCR calculated absolute TREC copy numbers to be 302.26, 127.72, 79.29, 36.69, and 24.09 TREC copies. As a sample matrix, newborn dried blood spots present challenges to sample preparation for digital PCR. However, these challenges can be minimized in order to utilize the advantages of digital PCR to provide insight into accurate TREC copies per sample without the added complication of standard curve calculations. In addition, digital PCR may provide improved resolution of low TREC copy numbers and allow discrimination of other primary immunodeficiencies, such as 22q deletion syndrome, from leaky or atypical SCID.

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**Summary**

T-cell receptor excision circles (TRECs) are used as the target analyte for identification of infants at risk for severe combined immunodeficiency (SCID). TRECs are generated during receptor editing for T-cells which is a normal process for lymphocyte maturation. SCID is characterized by low or absent T-cells,
which may be attributed to mutation in genes required for successful maturation. In addition, reduced T-cell numbers may result in attenuation of B-cell responses due to poor B-cell stimulation by T lymphocytes.

The TREC assay is the first molecular assay to be used as a primary screen to identify newborns with a genetic disorder. The TREC assay utilizes polymerase chain reaction (PCR) technology to amplify and detect TREC sequences, if present within the newborn dried blood spot. Infants with a positive SCID screen will have few or no T-cells which makes accurate quantification of TREC difficult due to the TREC target approaching the limits of detection (LOD) for PCR. Poisson statistics describe the variation in detection of a single copy of a gene of interest distributed at random within a complex mixture.

In addition to the technical challenges of detecting TREC while approaching the LOD, different approaches in testing methodology as well as variations in equipment and reagents used for testing may introduce additional discrepancy across laboratories when calculating TREC copy numbers. Due to technical and laboratory variation in TREC testing, nearly every newborn screening laboratory performing SCID screening utilizes a different action threshold for clinical intervention based on TREC copy number or Cycle threshold (Ct).

We attempted to determine absolute TREC copy number using a digital PCR approach which relies upon Poisson statistics to calculate target copy numbers without the need for a standard curve. We prepared and analyzed TREC plasmid samples, laboratory-derived dried blood spot calibrators, and newborn dried blood spot samples.

Digital PCR requires a separate DNA extraction as compared to the in situ TREC assay approach used by our laboratory. To closely approximate our in situ assay for use in digital PCR, 2.0 mm DBS punches were washed to remove inhibitors using the protocol developed for in situ amplification. Following DBS punch washing, 20 ul of 10 mM Tris-Cl buffer was added to washed DBS punches and the temperature cycled using the same thermal profile used for our in situ TREC assay (45oC for 5 min, 95oC for 20 min, and 45 cycles of 95oC for 15 secs and 60oC for 1 min). Following temperature cycling, the DNA extract was removed and transferred to a clean 96-well plate.

Preparation and loading of digital PCR chips was performed using the manufacturer’s protocol and required reagents (Life Technologies). TREC primer and probe sequences were those used for our in situ assay. Following loading of master mix with TREC primer and probe, chips were filled with immersion fluid and chips sealed using UV glue. Loaded chips were cycled according to manufacturer’s protocol and fluorescence read after chips were allowed to equilibrate to room temperature for 10 minutes.

Analysis of known 40 and 10 copy TREC plasmid standards by digital PCR yielded 41.61 and 10.53 copies. Further, comparison of digital data to in situ TREC assay data from lab-derived calibrators returned comparable results (60.59 copies vs 70.88 copies and 11.37 copies vs 6.18 copies). Newborn DBS samples were selected based on in situ TREC assay results and spanned Ct values from approximately 28.50 to 32.50, in 1.0 Ct increments. Digital PCR calculated absolute TREC copy numbers to be 302.26, 127.72, 79.29, 36.69, and 24.09 TREC copies. As a sample matrix, newborn dried blood spots present challenges to sample preparation for digital PCR. However, these challenges can be minimized in order to utilize the advantages of digital PCR to provide insight into accurate TREC copies per sample without the added complication of standard curve calculations. In addition, digital PCR may provide improved
resolution of low TREC copy numbers and allow discrimination of other primary immunodeficiencies, such as 22q deletion syndrome, from leaky or atypical SCID.

Since the TREC assay attempts to quantify copy numbers at the limit of detection for PCR, extrapolation of copy numbers through a standard curve adds additional variation in reported copy numbers. Digital PCR does not require a standard curve and has the potential to eliminate variation between reagents and instrumentation that are inherent in the TREC assay through the use of Poisson statistics. Additional experiments are currently underway to characterize TREC copy number results obtained using various approaches to the methodology of the TREC assay; a separate DNA extraction as compared to an in situ TREC assay.

A Multiplex Assay for Concurrent Newborn Screening of Spinal Muscular Atrophy (SMA) and Severe Combined Immunodeficiency (SCID)

F. Lee¹, J. Taylor¹, G. Yazdanpanah¹, M. Liu², C. Sun²; ¹Centers for Disease Control and Prevention, Atlanta, GA, ²Biogen Idec, Inc., Cambridge, MA

Abstract

Spinal muscular atrophy (SMA), the most common lethal autosomal recessive disorder in infants, is a motor neuron disorder caused by deletion or conversion of the SMN1 gene. The birth prevalence of SMA is 1:6000-10,000, with a carrier rate of 1:40. Recently developed therapies have shown promising results in symptomatic children enrolled in current clinical trials. Because of the pathogenesis of this disease, it has been suggested that for optimal outcome, therapy should start soon after birth and before symptoms develop, which would require newborn screening for the genetic defect. There were two major considerations in developing an SMA screening test. It is preferable to use an assay platform that has been established in many newborn screening laboratories. This would lower the capital cost and ensure that the throughput would be adequate. It would also be desirable if the SMA test can be multiplexed within an existing assay, so the additional material and labor cost to introduce this new condition for testing would be minimized. Since 2011, the implementation of screening for SCID has been growing rapidly among the newborn screening programs. The vast majority use a T-cell receptor excision circle (TREC) assay based on real-time PCR. We have incorporated the SMN1 target into our current TREC assay for SCID. By using a SMN1 Taqman probe based on the novel locked nucleic acid (LNA) technology, we have eliminated cross-reactivity to the SMN2 gene. Test results demonstrated excellent specificity for SMA, and the modification did not affect the quantitative results of TREC in dried blood spots (DBS) prepared from 150 cord blood samples. Clinical validity was provided by a double blind test of 26 donor samples, which correctly differentiated all 11 patients from the 15 parental carriers, with the TREC results appropriate for the ages of individual donors. The inclusion of the SMA screening reagents only adds an extra three cents to the current TREC assay.

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Next Generation Sequencing of CFTR from Dried Blood Spots Using the ION Torrent PGM™
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Abstract

Objective: Evaluate the performance of Ion Torrent Next Generation (Next-Gen) sequencing of the CFTR gene from DNA extracted from dried blood spot (DBS) and evaluate the concordance between the Ion Torrent and Sanger sequencing data.

Background: In order to characterize quality assurance samples used for CF mutation detection, CDC employs targeted Sanger sequencing of coding regions, intron-exon splice junctions and noncoding regions known to contain mutations. This assay requires amplification of 42 reactions followed by 86 sequencing reactions (covers 28 target regions). To increase the efficiency and lower the cost, a study to evaluate targeted Next-Gen sequencing was performed to determine whether the targeted CFTR Sanger sequencing assay could be replaced with an Ion Torrent Next-Gen sequencing assay.

Methods: DNA was extracted from one 3mm DBS punch using the Qiagen Generation solutions from 76 CFTR mutation carrying samples. Targeted regions of the CFTR gene were amplified using a custom AmpliSeq panel from Thermo Fisher. The Library prep and sequencing were performed and the data was analyzed using the Variant Caller plug-in with a Hotspot file consisting of 240 unique variants. Analyzed data was then compared to historic Sanger sequencing data.

Results: There was 100% concordance, excluding IVS8 TGxTy and 4700 8/9T, for the detected mutations and variations once Ion Torrent false positives calls and missed Sanger calls due to primer overwriting were filtered out. Complex small deletions and insertions required manual visualization to confirm calls.

Conclusion: Next-Gen sequencing for CFTR gene analysis using the Ion Torrent PGM platform shows potential for replacing the more expensive and labor intensive Sanger sequencing. The technology has made progress in addressing homopolymer tracks (sp.) with new enzyme development but regions such as IVS8 and 8T/9T may require Sanger confirmation.

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Summary

CDC’s Cystic Fibrosis (CF) DNA Proficiency Testing program began as a pilot program in early 2007. In order to fully characterize quality assurance samples used for CF mutation detection, CDC employs targeted Sanger sequencing of coding regions, intron-exon splice junctions and noncoding regions known to contain mutations. Sanger sequencing characterization requires the amplification of 42 reactions, followed by 86 sequencing reactions per sample, which covers 28 target regions consisting of approximately 18 kilobases of sequence. In an effort to increase the efficiency and lower the cost of sequence characterization, targeted next-generation sequencing (NGS) of the CFTR gene was evaluated to determine the feasibility of replacing targeted Sanger sequencing with the Ion Torrent PGM™ NGS system and Ion AmpliSeq™ CFTR Panel.

The goal of this study was to assess the sequence characterizations generated by the Ion PGM™ for the CFTR gene using DNA extracted from dried blood spots and comparing the data with traditional Sanger
sequencing data. DNA was extracted using a lysis method that is commonly used in NBS laboratories. This method was selected to determine the robustness of the assay on DNA of lower quality and quantity.

There are several advantages to using the Ion PGM™ method over Sanger sequencing including: 1) scalability, 2) ability to highly multiplex, 3) massive parallel sequencing, 4) automated analysis; and 5) cost savings. The Ion Torrent™ offers three different semiconductor sequencing chips with scaled density, providing increased flexibility in the number of samples one chooses to analyze for a given sequencing run. The ability to multiplex many samples allow for a substantial time and potential cost savings over traditional Sanger sequencing’s single sample processing. In addition, the Ion Torrent™ sequence data is easier to evaluate for insertions and deletions compared to Sanger sequencing since Sanger sequencing is impacted by frameshifts. Data analysis on the Ion Torrent™ is also automated which allows for a shorter analysis time.

DNA was extracted from one 3mm DBS punch using the Qiagen™ Generations Solutions 1 and 2 from 108 samples with at least one CFTR mutation. In preparing the libraries for sequencing on the Ion PGM™, targeted regions of the CFTR gene were amplified and barcoded using a custom Ion AmpliSeq™ CFTR Panel and IonXpress barcodes from Life Technologies/Thermo Fisher Scientific. The input genomic DNA per amplification pool was 5ng rather than the manufacturer-recommended 10ng per amplification pool. This decreased amount was due to the lower concentration of DNA that is usually obtained with the lysis DNA extraction method. The library preps were then pooled together, amplified, enriched, and sequenced according to manufacturer’s instructions. Data from the Ion PGM™ was processed and aligned to the human genome reference sequence (hg19). Variants were called and annotated using the Variant Caller plug-in with a customized Hotspot file consisting of 240 unique variants. The Hotspot file contains position information on variants and instructs the Variant Caller to include those positions in the output file in addition to any novel variants found. All analyzed data from the Ion PGM™ was then compared for concordance against the Sanger sequencing data.

The Ion AmpliSeq™ CFTR sequencing assay detected 162 mutations, as well as 535 variants excluding homopolymers such as IVS8. Overall, there was very good concordance. The precision for both mutation and variant detection as well as the sensitivity for mutation detection was 100% and the sensitivity of the variant detection was 99.3%. When the software could not make an automated call, the sequence was manually examined and addressed as follows. First, one sample was flagged for follow-up for the 2184insA mutation with a low allele frequency of 10%. The 2184insA mutation is in a poly-A region consisting of 7 Adenine residues. Since this technology is known to have difficulty reading through long homopolymers, a report of a 10% allele frequency would not be consistent with a true positive but requires Sanger sequencing confirmation. A second technical problem occurred with a F508del/F508C compound heterozygote sample. The analysis software correctly called the F508C variant; however the F508del mutation was classified as a no-call. A no-call is assigned to positions when the software cannot automatically make a determination of a particular mutation. Examination of the Ion PGM™ sequence data showed that both delF508 mutation and F508C variants were present in the sequence. This issue is likely to be fixed by a bioinformatic coding change in the algorithm used for analysis. Third, four samples received no-calls by the automated analysis for the 4575+1251C/T variant. These four no-call instances were due to either low sequence coverage, or strand bias due to the position of the variant near the end of an amplicon. This issue could be resolved by increasing the read coverage, either by loading fewer samples on a single chip, or re-designing the library pools to include another amplicon to cover this region. Since the 4575+1251C/T variant, located in the 3’ UTR, is a non-
CF causing variant, it would likely not be worth the increased cost associated with decreasing sample throughput or re-designing the assay.

The Ion Torrent™ sequencing workflow and subsequent automated analysis is more efficient than Sanger sequencing when sequencing greater than 8 samples. The Ion PGM™ is scalable in that the company offers different chip sizes for lower or higher sample throughput. The precision and sensitivity of the Ion PGM™ is greater than 99% for both variants and mutations, excluding the IVS8 repeat region. Since the Ion AmpliSeq™ CFTR Panel assay was designed to only identify the clinical relevant IVS8-5T, it cannot accurately distinguish the upstream TG repeat nor a 7T from a 9T. Next Generation sequencing for CFTR gene analysis using the Ion Torrent PGM™ platform could replace the more expensive and labor-intensive Sanger sequencing as a primary method, however Sanger sequencing should be used to fully characterize the IVS8 region.

NC NEXUS: North Carolina Newborn Exome Sequencing for Universal Screening Project
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Abstract

Since newborn screening (NBS) began in the 1960’s, technological advances have enabled its expansion to include an increasing number of disorders. Recent developments now make it possible to sequence an infant’s genome relatively quickly and economically. Clinical application of next generation or massively parallel sequencing is expanding at a rapid pace but presents many challenges. Its utility in NBS has yet to be demonstrated and its application in the pediatric population requires examination, not only for potential clinical benefits, but also for the unique ethical challenges it presents. With the overall charge “to explore the implications, challenges and opportunities associated with the possible use of genomic sequence information in the newborn period” the US National Institutes of Health has funded four centers through a U-19 cooperative agreement.

The University of North Carolina at Chapel Hill project, North Carolina Newborn Exome Sequencing for Universal Screening (NC NEXUS), will be studying two groups of patients: 200 with diagnosed conditions and 200 in a “well-child” group whose parents will be recruited prenatally. We will examine the sensitivity of whole exome sequencing in detecting pathogenic mutations in genes associated with targeted conditions which will be confirmed in a CLIA lab. Through a semi-quantitative metric scoring process we are developing a “Next-Gen Newborn Screening Panel” that will include additional conditions not currently detectable through standard newborn screening methods but with onset in childhood and medically actionable. The presentation will provide an overview of the main project activities, give examples of the scoring process for genes, and describe the proposed parental decision-making protocol and tools under development for return of unanticipated or "incidental" findings.

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Summary

What has been termed “Next-Generation Sequencing” (NGS) or massively parallel sequencing, the use of high-throughput sequencers with the ability to analyze all base pairs in an organism in a relatively short period of time as opposed to previously used methods such as Sanger sequencing, is opening the possibility of expanding the number of conditions that can theoretically be screened for in an individual to his or her entire genome. The most commonly used NGS is whole exome sequencing (WES), in which only the protein coding parts of genes, known as exons, are sequenced. These make up approximately 1% of a person’s genome. Barriers to adding conditions to recommended newborn screening panels due to lack of a testing method will now be overcome as long as there is a known genetic etiology for a disorder. In the list of twenty-three conditions considered but not included in the original recommended newborn screening (NBS) panel because they lack an accurate screening method (Watson et al. Pediatrics 17, S296, 2006), 21 are detectable in some or all cases with molecular genetic analysis and, therefore, have the potential to be added to NBS panels. While studies are underway to examine the use of NGS in clinical practice, screening a newborn with WES raises additional complexities. The newborn lacks the autonomy of an adult who decides to undergo screening through WES to determine whether they have a gene mutation that can predispose them to developing a condition such as cancer. In NBS these decisions are made by and directly impact the parents, an additional complication that requires special consideration.

With these challenges in mind, pilot projects to examine these issues are being funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and the National Human Genome Research Institute (NHGRI) of the National Institutes of Health under the Genomic Sequencing and Newborn Screening Disorders research program. The University of North Carolina at Chapel Hill is one of the grantees.

To ultimately determine the clinical utility of NGS in NBS and evaluate whether such an approach offers added value, it will be imperative to assess the sensitivity and specificity of WES for currently screened conditions. Will NGS provide diagnostic data as accurate as currently utilized screening methods such as tandem mass spectroscopy? Will these data significantly augment our ability to predict disease prognosis and enable more targeted management? To answer these questions, we will have one cohort of 200 patients from age 0-5 years with diagnosed conditions. These will include four of the most common conditions detected by current newborn screening: PKU, MCADD, CF and hearing loss. Also in this group will be children with conditions that meet criteria for newborn screening but heretofore were not possible to detect due to lack of an adequate screening method. These will include lysosomal storage disorders and primary ciliary dyskinesias. The application of WES in these cohorts will allow not only the delineation of the causative mutation in the proximally causative gene, thus augmenting studies of phenotype-genotype relationships, but it will also create a valuable long-term resource for researchers to investigate how currently unknown loci contribute to clinical heterogeneity.

Another goal of our study is to develop a “Next-Generation Newborn Screen” that will include genes associated with conditions that are part of the current recommended uniform screening panel as well as those determined to fulfill criteria for newborn screening that may be detected with WES. These will include, for example, genes associated with early childhood cancer such as multiple endocrine neoplasia type IIB due to mutations in the RET gene. All such conditions will have onset in childhood and be medically actionable, meaning that early identification will allow prevention or amelioration through screening or early treatment.

Incidental findings are clinically significant mutations in genes detected through WES that were not the original intent of the testing. The American College of Medical Genetics and Genomics has recommended that mutations in 56 genes be reported back to patients unless they opt out of receiving such findings. The list of these 56 genes was not developed specifically for children. Interpretation of a gene variant in the context of a newborn is very different as compared to an adult who has passed the age when manifestation of that genetic change would be apparent. These genes can be categorized differently, depending on the age of onset and the degree of actionability. Even those conditions that are not treatable in the traditional sense, such as those associated with intellectual disabilities like Angelman syndrome, may benefit children and their parents by being identified early, leading to earlier enrollment in developmental intervention services and avoidance of the diagnostic odyssey. There is a category of adult-onset medically actionable conditions such as risk of breast cancer due to BRCA1 mutations. In order to investigate the use of WES in a typical group of infants, we will include a cohort of 200 healthy newborns whose parents will be recruited and consented during pregnancy. Our study will randomize both cohorts into two groups, one whose parents will only receive NGS-NBS results (and results related to their disorder in those with known conditions) and another whose parents will have the option of receiving additional findings in the other two categories of conditions (childhood onset not medically actionable and adult onset medically actionable). We also plan to give parents the option to learn results of carrier status in the infant for clinically significant autosomal recessive or X-linked disorders. This will be done with the use of a decision aid, an online tool that is also being developed as another part of our study. Parental attitudes about being given a choice to receive additional genomic information about their child, choices made and the impact of this information will be studied.