Complete Title: NC NEXUS: North Carolina Newborn Exome Sequencing for Universal Screening

Short Title: NC NEXUS

Drug or Device Name(s): N/A

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1. Clinical Protocol

Title of Clinical Protocol

Short Name: The NC NEXUS Study
Long Name: The North Carolina Newborn Exome Sequencing for Universal Screening Study

Study Design

General Study Design

The NC NEXUS project is an exploratory longitudinal cohort study, with randomization of enrolled parents into two groups with different opportunities for decision-making about categories of non-medically actionable genomic information.

Cohorts:

We will enroll parents and their eligible child into the following 2 cohorts:

The "Diagnosed Cohort": Our goal is to sequence 200 children from this cohort including infants and children up to age 5 who have metabolic disorders such as phenylketonuria (PKU) and medium chain acyl-CoA-dehydrogenase deficiency (MCADD); cystic fibrosis (CF) and *CFTR*-related metabolic syndrome (CRMS); congenital hearing loss; primary ciliary dyskinesia, and a variety of other rare conditions.

The "Well-Child Cohort": Our goal is to sequence 200 infants whose parents enroll prenatally. The expectant mothers must be pregnant with an intrauterine pregnancy of 18 weeks or greater, have no pending or positive prenatal diagnostic test results for congenital malformations or chromosomal abnormalities and have been identified by medical personnel in the obstetrics clinic as possible candidates.

We will consider requests from couples who receive their OB care elsewhere but who have had consultations with the UNC prenatal clinic on a case-by-case basis.

Randomization:

In order to assess the impact of the additional non-medically actionable genomic findings available upon request, parents will be randomized in a 2:1 ratio to "Decision" or "Control" groups, respectively. Both groups will learn any childhood onset medically actionable results ("NGS-NBS") from genomic sequencing at their return of result visit.

Study design narrative:

The NC NEXUS project will utilize a system of tiered informed consent by which parents will participate in a study of informed decision-making about whether they would accept

NGS-NBS for their child or infant, and (in the case of those randomized to the "Decision" group) whether they wish to learn about other additional categories of genomic information.

Potential participants will be approached by a recruiter during a regularly scheduled clinic visit and provided with an informational brochure about the NC NEXUS study (NEXUS Recruitment Brochure for the Diagnosed cohort: Appendix 9 and NEXUS Recruitment Brochure for the Well-child cohort: Appendix 10).

If they are interested in learning more about the study, contact information will be obtained and they will be given a study brochure and consent form.

There will be no further interactions with parents who decline to learn more about the study

Parents who expressed interest in the study will receive a telephone call during which the informed consent for initial participation will be reviewed and consent given verbally (NCNEXUS_information_sheet_Phase_I_Diagnosed cohort: Appendix 11 and NCNEXUS_information_sheet_Phase_I_WC cohort: Appendix 12).

Those who agree to participate will be given access to the online decision aid, which will provide information about genomic sequencing and the potential types of results that would be included in the "NGS-NBS" analysis (NC NEXUS Decision aid overview: Appendix 13; NEXUS Online DA Decision 1 Shooting Script: Appendix 14; NEXUS Online DA Shooting Script QA content: Appendix 15).

Those who are not interested in participating will complete an exit questionnaire for decliners.

After receiving additional information through the electronic decision aid, parents who are interested in obtaining sequencing for their child will be scheduled for an in-person study visit ("Visit 1") with a genetic counselor to obtain formal consent for sequencing (NC NEXUS_consent_phase II_diagnosed cohort: Appendix 16 and NC NEXUS_consent_phase II_Well-Child cohort: Appendix 17). Cheek swab samples will be delivered to the BSP and MGL for processing. Exome sequencing will be performed, with focused informatics analysis depending on the cohort (described below).

The randomization status will be revealed to the parents when they are scheduled for their return of results. Parents who are randomized to the "decision" group will be given access to additional content in the electronic decision aid prior to their second in-person study visit (NEXUS_Online DA_Decision 2_Shooting script: Appendix 18). This information will include a description of the additional categories of non-medically actionable

information.

Couples from the well-child cohort who are randomized to the control group and who have negative NGS-NBS results, may learn these results by a scheduled phone call with a genetic counselor/clinical geneticist.

Couples from the well-child cohort who are randomized to the decision group and are requesting results from the carrier status category will be able to learn these results during a scheduled phone call with a genetic counselor. If they have requested either or both of the other two categories of additional information and these are negative, all of their additional results may be disclosed during a scheduled phone call with a genetic counselor/clinical geneticist.

All participants with clinically relevant positive results will have a second in-person study visit ("Visit 2") with a board certified medical geneticist and genetic counselor for return of results from NGS-NBS and (in the case of the "Diagnosed Cohort") the indication-based analysis, and for the other two additional categories of information.

Parents who are randomized to the "control" group will receive their primary results but will not be eligible for additional categories of information.

Parents who are randomized to the "decision" group will receive their primary results and will have the opportunity to discuss any questions they have regarding additional categories of non-medically actionable information. They will then be eligible to request analysis of any, all, or none of the additional information.

Parents randomized to the "decision" group will have up to one additional visit with a medical geneticist and/or genetic counselor ("Visit 3") depending on the additional categories of non-medically actionable information they have requested.

Parents will be asked to complete questionnaires at defined time points during the study (see study design schematic below). Parents who complete the questionnaires will be paid $20 for each questionnaire completed. Payment will be mailed immediately upon completion of a questionnaire. We have found that this amount of money recognizes their time and effort but is not coercive. Measures included in these questionnaires are shown in NC NEXUS Project 3 Longitudinal Study Measure (see Appendix 19). Slight changes or adjustments based on feedback from user testing may lead to minor changes (e.g., to reduce burden by removing some items or measures, change wording if any is found to be confusing to users, or add measures if users suggest we are missing a key construct), in which case the FDA will be provided with a 5-day notice of any updates or changes in the measures.

Intake Form (Draft version will be provided upon request): The

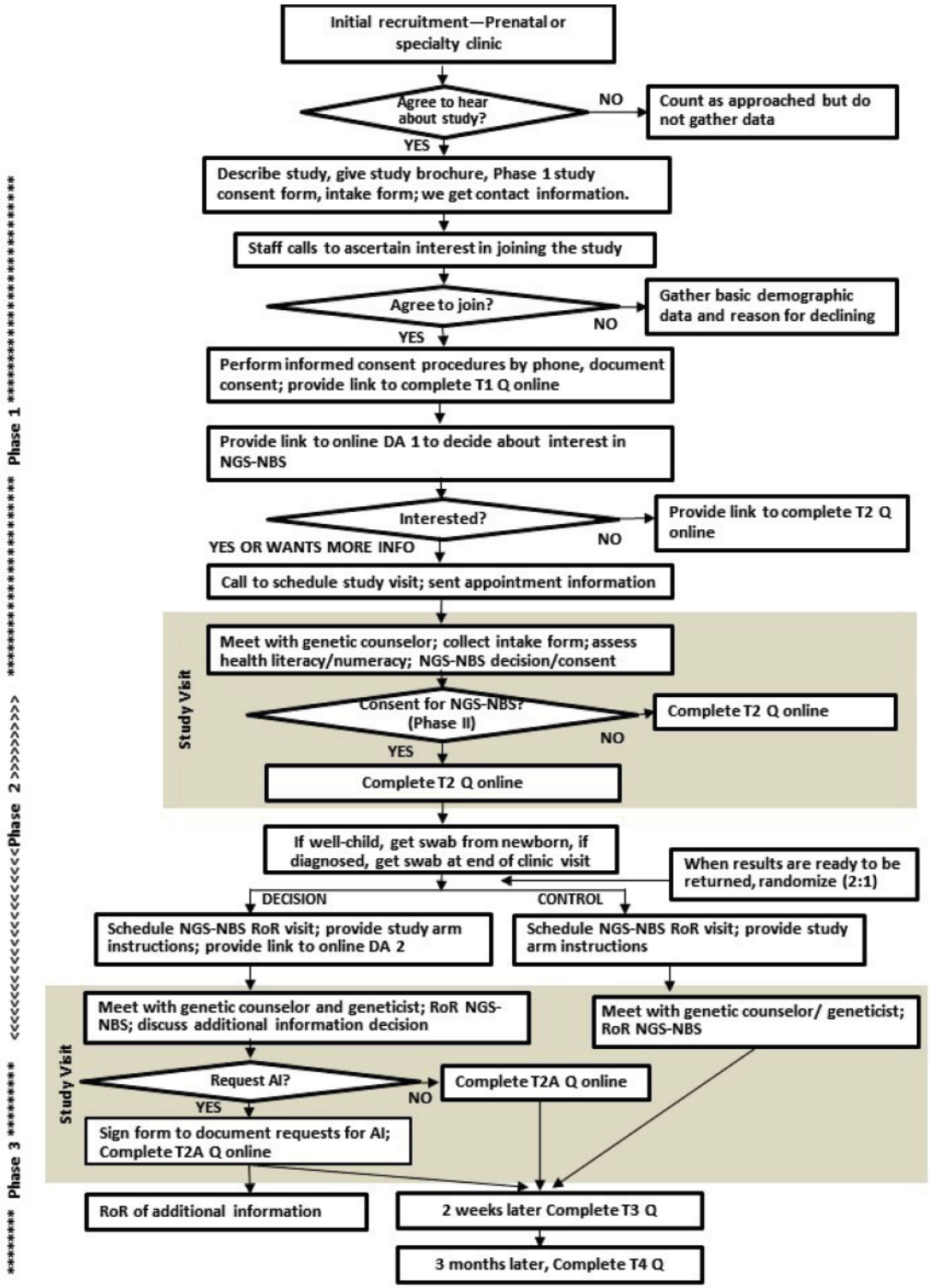
intake form collects information about demographics, previous experience with genetic testing, knowledge about genomic sequencing, and (for the "Well-Child" Cohort) pregnancy anxiety. Parents will be asked to complete this form and bring it with them to the initial study visit, if they agree to a visit.

Time 1 questionnaire (Draft version upon request): After parents decide to proceed with an in-person study visit (either to consent to NGS-NGS or to get more information about it in an in-person consultation) or not to proceed with an in-person visit (declining further participation in the study), they will be given access to the Time 1 questionnaire, which will provide valuable information about differences between parents who are and who are not interested in NGS-NBS.

Time 2 questionnaire (Draft version will be provided upon request): All parents who attend the initial in-person study visit (whether or not they have accepted NGS-NBS) will complete the Time 2 questionnaire, which gathers data about their decision-making process, mood, and knowledge about the project. In addition, parents in the "decision" group will be asked to complete a brief post-decision questionnaire (the Time 2A questionnaire) after they decide whether or not to request additional genomic findings to assess the consequences of having this option.

Follow up Assessments (Time 3 and Time 4 questionnaires) (Draft versions will be provided upon request): All parents will complete two follow up questionnaires following the return of result visit: a Time 3 questionnaire completed within 2 weeks of the visit and a Time 4 questionnaire completed 3 months after the visit. These questionnaires will assess the short- and longer-term consequences of decision-making and results disclosure.

Study Design Schematic Figure


Phase 1
Phase 2
Phase 3
Initial recruitment—Prenatal or specialty clinic
Agree to hear about study?
NO
Count as approached but do not gather data
YES
Describe study, give study brochure, Phase 1 study consent form, intake form; we get contact information.
Staff calls to ascertain interest in joining the study
Agree to join?
NO
Gather basic demographic data and reason for declining
YES
Perform informed consent procedures by phone, document consent; provide link to complete T1 Q online
Provide link to online DA 1 to decide about interest in NGS-NBS
Interested?
NO
Provide link to complete T2 Q online
YES OR WANTS MORE INFO
Call to schedule study visit; sent appointment information
Study Visit
Meet with genetic counselor; collect intake form; assess health literacy/numeracy; NGS-NBS decision/consent
Consent for NGS-NBS? (Phase II)
NO
Complete T2 Q online
YES
Complete T2 Q online
If well-child, get swab from newborn, if diagnosed, get swab at end of clinic visit
When results are ready to be returned, randomize (2:1)
DECISION
CONTROL
Schedule NGS-NBS RoR visit; provide study arm instructions; provide link to online DA 2
Schedule NGS-NBS RoR visit; provide study arm instructions
Study Visit
Meet with genetic counselor and geneticist; RoR NGS-NBS; discuss additional information decision
Meet with genetic counselor/ geneticist; RoR NGS-NBS
Request AI?
NO
Complete T2A Q online
YES
Sign form to document requests for AI; Complete T2A Q online
RoR of additional information
2 weeks later Complete T3 Q
3 months later, Complete T4 Q

2 Study Procedures

Participant Selection

Participants can be either sex and of any race or ethnicity, but parents must be fluent in English or Spanish. For mothers who are married or in a marriage-like relationship, their partners must also consent to participate. Participants will self-identify their preferred language (English or Spanish)

Although this study will focus on newborns, in order to increase our numbers of prospective subjects in the "Diagnosed Cohort," we will enroll children up to age 5 years. In order to obtain more meaningful data from WES analysis, particularly for conditions such as PKU, including determining any genotype-phenotype correlations or influence of other genetic factors, it is important to obtain clinical data in children beyond infancy. The "Well-Child Cohort" will consist of newborns in order to more accurately reflect the typical age of NBS.

Pregnant women and their partners will be recruited from the Prenatal clinics at UNC and verification of their pregnant status will be by self-identification and by determining that they are being followed in the prenatal clinics and have had their pregnancies verified as part of standard prenatal care.

Anticipated Number of Research Subjects

Recruitment will use established channels at UNC, and we expect that ~80% of mothers we approach for recruitment will have a partner who is reasonably available and who therefore would be approached for recruitment.

For the "Diagnosed Cohort," there are 560 current pediatric patients under age 5 with the selected conditions being followed at UNC, and an additional 330 infants under 6 months old are diagnosed each year. We estimate that ~80% of parents of children in the "Diagnosed" cohort will elect to have their child undergo sequencing. Thus, in order to sequence 200 children in this cohort, we anticipate enrolling 250 family units (couples or single parents, and their child).

For the "Well-Child Cohort," there are approximately 3,500 expectant mothers at UNC per year who will be eligible, providing a large population from which to recruit. In our prior study on newborn screening for fragile X syndrome (FXS), 64% of couples agreed to join the study and accepted screening. We therefore estimate that ~64% of parents approached for recruitment to the "Well-Child Cohort" will agree to join it, although this estimate is conservative because joining our study will not necessitate also accepting NGS-NBS. We project a 5% dropout rate between Times 1–3 (when parents are actively involved in making decisions and receiving results) and an additional 5% dropout at Time 4. In order to sequence 200 children in this cohort, we anticipate enrolling 350 eligible family units.

Based on these estimates, we will approach 48 parents per month over 20 to 21 months for recruitment to yield a sample of 400 completing the study over approximately 30 months (200 each in the diagnosed cohort and well-child cohort).

Inclusion Criteria

"Diagnosed Cohort"

Parents meeting the following criteria:

1. Parents of a child who meets the criteria below AND
2. At least 18 years old.
3. For mothers who are married or in a marriage-like relationship, their partners must also consent to participate. Mothers who are not married or not in a marriage-like relationship will be able to participate individually.
4. Must be able to provide informed consent for their child and for themselves
5. Must be fluent in English or Spanish

Children meeting the following criteria:

1. Infants and children from 0-5 years
2. Diagnosed with known or suspected monogenic disorder, such as:
   - Phenylketonuria
   - Medium chain acyl-CoA-dehydrogenase deficiency (MCADD)
   - Cystic fibrosis or *CFTR*-related metabolic syndrome.
   - Congenital hearing loss
   - Other rare disorders such as primary ciliary dyskinesia or mucopolysaccharidosis

   OR
   - Those with positive newborn screens but non-confirmatory follow-up testing ("false positives")
3. Medically stable

"Well-Child Cohort"

Parents meeting the following criteria:

6. Pregnant with an intrauterine pregnancy of 18 weeks or greater
7. At least 18 years old
8. For mothers who are married or in a marriage-like relationship, their partners must also consent to participate. Mothers who are not married or not in a marriage-like relationship will be able to participate individually.
9. Must be able to provide informed consent for their child and for themselves
10. Must be fluent in English or Spanish
11. Have no pending or positive prenatal diagnostic test results for congenital malformations or chromosomal abnormalities
12. Have been identified by medical personnel in the OB clinic as possible candidates.
13. Couples recruited into the Well-Child cohort who deliver at an outside location (not

at UNC Hospitals) will be allowed to participate. Couples who are not patients of the prenatal clinic but who have consulted with them for services such as ultrasounds and who contact us asking to participate may come to the Womens' Hospital and be recruited into the study by the prenatal recruiter.

Newborns:

1. Have no complications at the time of birth or unexpected medical problems; however, depending on their clinical course, those whose parents have previously consented to the study may have DNA sampling once stabilized and discharged from the Neonatal Intensive Care Unit, if the parents agree.

Exclusion Criteria

Parents:

1. Younger than 18 years old
2. Unwilling to complete study procedures
3. Have cognitive or other impairments that preclude them giving informed consent
4. Disagree about their child's participation
5. Transfer their prenatal care to another institution
6. Are not fluent in English or Spanish

Children:

1. Do not meet diagnostic criteria as above
2. Medically unstable
3. Medical care transferred to another institution
4. Not born at UNC Hospitals in Chapel Hill, NC

Recruitment and enrollment procedures

After having been identified by their clinician as eligible, couples will be contacted by a study recruiter, and asked if they would like to hear about the study. Fathers will also be recruited if they are reasonably available. Thus, these procedures discuss the involvement of "parents." However, when fathers are not reasonably available, mothers may participate on their own. For those in a couple relationship parents must be concordant in their decisions to consent or the couple will not be eligible.

The "Well-Child Cohort":

Initial Contact: The recruiter will approach those pregnant couples in the UNC prenatal clinic who have been identified as potentially eligible and who have been informed about the study by their clinician.

- **Not eligible:** We will thank them for their time, not gather any information, and not contact them again.
- **Decliners**: We will thank them for their time, not gather any information, and not contact them again.
- **Accepters**: The recruiter will briefly describe the study and give the couple a

study brochure (NEXUS Recruitment Brochure for the Well-child cohort: Appendix 10) that describes study procedures, genomic sequencing, the kinds of conditions that could be identified, the randomization process, and a decision aidto help them make a decision about joining the study. They will also be given a copy of the consent form (NEXUS Recruitment Brochure for the Well-child cohort: Appendix 10) about joining the study to read and the intake form for each member of the couple (or just for mothers if they are participating without a partner). The intake form collects information about demographics, previous experience with genetic testing, knowledge about genomic sequencing, and pregnancy anxiety. Couples will be asked to complete this form and bring it with them to the study visit.

The couple will be asked if they will agree to being contacted by phone by the study scheduler to ascertain their interest in joining the study. Couples who agree will be asked for their phone number and contact information, including email address, and their language preference, which will be entered into the study database (REDCap) along with the clinic from which they were recruited and gestational age of the pregnancy. The recruiter will provide a timeframe for the call (e.g., a week) and ask that both members of the couple read the study brochure and informed consent form before that time and be present on the call.

For those who agree to join, the recruiter will offer access to the first questionnaire (T1Q) in either paper form or electronic links. iPADS will be available for use by those who do not have a smart phone or other device. If both members of the couple are present and have time, they can also access the first decision guide. Alternatively, if participants wish to wait until they leave the clinic, or for times when the partner is not present, study staff will email the links to the T1Q (see below).

Participants will also be sent links to the first electronic decision guide; these links become active after the completion of the T1Q. For couples, each parent will complete the questionnaire independently. The link will take them to a copy of the information page that will instruct them that by proceeding to the questionnaire, they will have agreed to join Phase I of the study.

Recruitment phone call: The study scheduler will call the couple and ask if they would like to join the study.

- **Decliners**: Couples who indicate they are not interested in joining the study will be asked to provide basic demographic data and a reason for declining. Completion of this step will end their participation and their identifying information will be shredded.
- **Accepters:** We will obtain the **couple's verbal consent** for Phase 1; joining the study.

If both parents have not read the study brochure and the consent form by the time the scheduler calls, they will be given additional time to read it, agreeing on how much time that will be.

Parents who have access to an Internet-enabled computer will then be provided with a link to access the Time 1 questionnaire. Each member of the couple will complete the questionnaires in the study independently.

After completing the Time 1 questionnaire, they will be given access to the online electronic decision aid. The decision aid will provide information about sequencing and the types of results available, the frequency of such findings, the risks and benefits of study participation, and guide parents thru the decision-making process. Parents will be encouraged to view the decision aid before the study visit and can use it to indicate their choice of one of three options:

(1) not interested and do not wish to schedule a visit, or
(2) interested and want to schedule a study visit, or
(3) undecided and want to schedule a study visit to learn more.

Thus, couples are **not** expected to make a decision at this time and will have ample time to gather information and consider their options.

Parents who do not have internet access will be mailed a copy of the Time 1 questionnaire with instructions to return the completed copy in a pre-paid envelope. They will then view the decision aid at the study visit.

Those who agree to a study visit will be scheduled and sent information about the appointment time, date and location of the visit.

- **Non-compliant decliners:** Couples who verbally consent to the study but who do not successfully schedule a study visit or who fail to appear for their scheduled study visit will be asked if they would be willing to reschedule their visit. If not, they will be considered to be decliners. Decliners will be asked to provide basic demographic information (for comparison to the accepters) and their reasons for declining**.** Completion of this step will end their participation in the study.

Study Visit Activities: Couples who schedule a study visit will meet in person with a genetic counselor who will collect their completed intake form, assess their health literacy and numeracy with a validated brief interview, and discuss any issues generated by the electronic decision aid about accepting or declining sequencing of their child. The counselor will answer questions and assess their understanding of main points of informed consent including the range of results that could be returned, the planned use and storage of genetic data as well as the risks and benefits of genomic sequencing**. Consent (for Phase II; sequencing) will be obtained** from both members of the couple except in cases where the father is not reasonably available. In those cases only the mother will be consented.

In cases when one parent is unable to attend the study visit, we will use Skype for the counseling and consent process. The Skype counseling and consent will be done in the CTRC and will be conducted by the clinical geneticist and/or genetic counselor. The partner who is unable to attend will sign the consent form during the encounter and return it to the study office.

- **Sequencing consenters**: After consenting, couples will complete the Time 2 questionnaire about their decision, mood, and knowledge about the project. They will also be asked to provide consent for access to their child's state newborn screening results and pertinent medical records.
- **Sequencing decliners**: Parents who decline sequencing will be asked to complete the Time 2 questionnaire and will exit the study.
- **Couples who are unsure about their sequencing decision:** The couple can defer their decision about sequencing and defer scheduling a study visit. This option will provide additional time for parents to view the decision aid and make a decision about genomic sequencing prior to giving birth (which normally occurs around 40 weeks gestation). In order to facilitate the time couples have to read the informed consent forms and view the electronic decision aid, we are providing access to these prior to and after the study visit. They will have ample time to have their questions answered, and confer with others before providing consent (e.g., family members, healthcare providers).
- If and when a deferring couple decides to proceed, they will contact the study office and a study visit will be scheduled (see above: study activities). The genetic counselor will review the information and obtain consent. After providing consent for sequencing, couples will complete the Time 2 questionnaire as described above. They will also be asked to provide consent for access to their child's state newborn screening results and pertinent medical records.

Couples can change their decision from yes to no before the sample is obtained after the baby is born. They will be asked about their decision, complete the Time 2 questionnaire again and exit from the study.

The "Diagnosed Cohort":

Many of these families live long distances from UNC and we would like to coordinate the study visit with an upcoming clinical visit which necessitates that we contact them before their child's appointment. All parents will have first been introduced to the study by their child's clinician either in person, by phone or by a letter accompanying the study pamphlet.

Initial Contact: Parents will be contacted by mail before their upcoming clinic visit and sent the study brochure and a letter from their child's clinician inviting them to participate with an opt-out postcard to return if they wish to decline. They will also be sent a copy of the consent form to join the study. If parents do not opt out, they will be contacted 2-4 weeks later by a telephone call or recruited at the time of their child's appointment.

Phone call recruitment and study visit scheduling: as described above

In-Person Recruitment: Some parents may not be reachable by phone before their child's clinic visit. In these cases, a study recruiter will ask them if they want to join the study at the time of their child's clinic visit. When possible, they will have already been sent the recruitment brochure and the consent form to join the study. If they wish to join,

they can give verbal consent for Phase I (joining the study) and complete the intake form. They will then complete the Time 1 questionnaire (online) and the rest of the study visit activities as described above. After they complete the Time 1 questionnaire, they will be provided with a link to the electronic decision aid.

Parents who attend a study visit (including those who are recruited in person) will meet with a genetic counselor to discuss any questions they have about NGS-NBS and about accepting or declining sequencing of their child as described for the "Well-Child" cohort above. Consent for Phase II (consent for sequencing) will be obtained from those who agree.

Decliners: As described above.
Sequence consenters: As described above.
Sequence decliners: As described above.

Study treatment and/or diagnostic procedures

Sample Collection and DNA isolation:

Saliva samples will be collected by trained study personnel using Oragene sponge collection kits. Sponges will be swabbed inside the cheeks and along the gums of the infant (Appendix 1).
Need to describe the procedures of collection of saliva (any known inferences such for foreign substances) and tracking/linking samples with participate.

- In the "Well-Child Cohort," arrangements will be made to contact us when the baby is born to obtain the sample. The PIs will be notified through EPIC at the time of the infant's delivery. The buccal swab will be obtained during the baby's stay at UNC Hospitals following his or her delivery or at a future well-baby appointment at a UNC clinic or at a postpartum clinic visit for the mother.

- In the "Diagnosed Cohort," the buccal swab will be obtained after parental consent to sequencing is obtained which could occur at the time of study visit or at a future in-person visit).

DNA will be isolated from duplicate samples using standard procedures in the UNC BSP and the CLIA-certified Molecular Diagnostic Laboratory (MSMI DNA extraction from OC-175 collection systems: see Appendix 2 and Mol Gen Newborn Saliva Extraction by BioRobot EZ1: see Appendix 3).

Exome Sequencing:

An aliquot from each uniquely coded DNA sample will be transferred by the BSP to the lab of Dr. Jonathan Berg, MD, PhD, Associate Professor in the Department of Genetics at UNC. Samples will be subjected to NGS using whole exome sequencing (WES) as described in section 2. (Agilent SSEL Automated Target Enrichments: see Appendix 5). Sequencing libraries will be transferred to the HTSF for massively parallel sequencing using the Illumina HiSeq 2000 or HiSeq 2500 platform (Appendix 6).

Changes in technology may alter the choice of target capture or sequencing platform, which might affect the yield of positive results but would not affect the nature of results returned.

Bioinformatics:

Raw sequence data from the HTSF will be analyzed using standard bioinformatics methods to map sequence fragments and align them to the reference human genome. Genetic variants will be identified using a custom pipeline that has been developed in collaboration with colleagues in the Department of Genetics and the Renaissance Computing Institute (RENCI). The current pipeline is as follows:

- Fragments are aligned against an indexed reference human genome (NCBI 37.1 / hg19) using BWA
- Resulting SAM files are sorted, indexed, and converted to binary BAM files using Picard and SAMtools.
- Post-alignment optimization, including PCR duplicate removal, realignment of reads, and quality score recalibration are performed using The Genome Analysis Toolkit (GATK).
- Single nucleotide variants and small insertions and deletions are called using the GATK Unified Genotyper.
- Quality metrics will be incorporated so that coverage with quality scores can be assessed for any given nucleotide.

Genetic variants identified (approximately 100,000 per individual exome) will be deposited in a dedicated database and will be extensively annotated and subjected to *in silico* analysis. Annotations that will be applied to the variants and reviewed by the analysts who interpret the variants are as follows:

- RefSeq transcripts, with protein effects
- 1000 genomes project and Exome Aggregation Consortium (ExAC) variant frequency data
- Human Gene Mutation Database (HGMD) mutations and ClinVar pathogenicity assertions
- Additional annotations are possible, such as dbSNP entry, OMIM identifiers, evolutionary conservation, Polyphen and other protein prediction algorithms.
- Curated references from the biomedical literature.

Categorization of possible genomic information:

In order to evaluate the range of genomic findings, we utilize a framework for "binning" genes into categorical lists that will facilitate informed decision-making. We will utilize the following categories of genomic results in this study:

1. ***Next-Generation Sequencing Newborn Screen (NGS-NBS):*** Medically actionable childhood conditions, representing the core results that will be returned to all participants in the study. The NGS-NBS includes genes implicated in conditions that are currently screened for in standard state newborn screens,

including metabolic disorders, endocrine disorders, and hearing loss. In addition, we will include other medically actionable conditions that are not amenable to current screening methods but can be detected using genetic sequencing (e.g. hereditary cancer susceptibility with onset or initiation of screening protocols in childhood). The criteria for determining which genes to include in the NGS-NBS are part of the overall aims of the research project (see below). These findings represent the default set of results that would be returned with every sequencing report. All parents consenting to sequencing of their child will learn if their child has one or more variants in this category that are determined to indicate with high likelihood that the child has or will likely develop a particular genetic disorder.

2. ***Additional genomic findings:*** Conditions that do not meet the threshold for inclusion in the NGS-NBS. Only parents randomized to the "decision" group will be asked to decide if they wish to learn any, all or none of these additional findings. Human curation and analysis of these variants will **not** be performed until parents request them. This analysis would **not** be done for children in the control group (see randomization procedure below). These additional findings fall into the following categories:

   A. *Medically-actionable adult onset conditions:* These disorders would be similar to the kinds of results described in NGS-NBS (above) but are related to conditions in which the onset or initiation of screening protocols occurs in adulthood, such as Hereditary Breast and Ovarian Cancer gene mutations.

   B. *Non-medically actionable childhood onset conditions:* The findings in this group relate to childhood health conditions that have no specific medical interventions. This category includes genes implicated in genetic disorders for which no specific preventive measure or treatment has been shown to mitigate morbidity. Examples include Rett syndrome and Angelman syndrome, conditions associated with intellectual disability in childhood for which there is no medical treatment, but for which early identification and initiation of therapy services are beneficial.

   C. *Carrier status:* This category relates to findings that have reproductive implications, such as carrier status for recessive disorders such as cystic fibrosis and Fanconi anemia.

3. ***Excluded genomic findings*** *(Non-medically actionable adult onset conditions):* In keeping with ethical norms in the field and to protect a child's ultimate autonomy, we have defined a process for choosing genes that would be excluded from analysis and would not be returned, regardless of the randomization status. Thus, **no participants will receive genomic results related to non-medically actionable adult onset conditions**. This category is exemplified by conditions such as amyotrophic lateral sclerosis (ALS).

Defining Clinical Actionability:

We have developed a semi-quantitative metric for scoring the actionability of gene-disease pairs in order to facilitate their assignment into "bins" used to guide the return of results (30). This framework has been adopted by the NC NEXUS project, and we have assembled a diverse group of experts and stakeholders to systematically assess genes implicated in Mendelian disease. This method assesses each gene-disease pair through the following five questions:

1) What is the nature of the threat to health for an individual carrying a pathogenic allele of the given gene? (Ranging from sudden death to no phenotypic impact)
2) What is the chance that this threat will materialize? (Related to penetrance)
3) How effective are interventions for preventing harm? (A critical component of medical actionability)
4) How acceptable are the interventions in terms of the burdens or risks placed on the individual? (Reflecting the possible hazards and downsides of medical intervention)
5) What is the knowledge-base regarding the nature of the disorder and its management in pre-symptomatic individuals?

Each gene-disease pair receives a score from 0 to 15, and we will determine a threshold level that indicates a level of medical actionability that justifies inclusion in the NGS-NBS.

In addition to the actionability score, each condition will be characterized in terms of the typical age of onset and age at which interventions would be initiated. Thus, we can generate a two-dimensional representation of the age-based actionability that can be used to define the four categories described above.

- Conditions that have an actionability score that exceeds the threshold and have onset of disease or interventions before age 18 would be considered candidates for NGS-NBS.
- Conditions that have an actionability score that exceeds the threshold but have onset of disease or interventions after age 18 would be included in the adult-onset medically actionable category. Many genetic conditions may have variable ages of symptom onset in either childhood or adulthood, such as Pompe disease, Krabbe disease, Fabry disease, or cardiac arrhythmias. For this reason, conditions such as these will be placed with childhood-onset conditions.
- Conditions that have an actionability score below the threshold but have onset before age 18 would be included in the childhood-onset non-medically actionable category.
- Conditions that have an actionability score below the threshold and onset after age 18 would be included in the adult-onset non-medically actionable category (and thus not eligible for return of results).

A list of conditions that have been scored by the NC NEXUS team as of the date of submission are provided in NC NEXUS_Actionability Scores (see Appendix 8). The tables in Appendix 8 document the initial work performed in the NC NEXUS project and previous work (Berg et al. 2015) to develop a semi-quantitative metric for assessing clinical actionability of gene-disease pairs. This list of 658 gene-disease pairs is a work in

progress and we expect to curate > 200 gene-disease pairs by the end of the NC NEXUS project. Scores, may be update periodically to reflect progress in the evidence base or advances in management of different genetic disorders. These scores will be used, in combination with curated information regarding the age of onset or age at which interventions would occur, to define the four categories of genomic information defined in this protocol. Since the development of final lists conditions in each of these categories is a primary outcome of the study, we anticipate that the work of binning each Mendelian disorder is expected to continue throughout the study period, and that periodic updates of the lists will occur. The FDA will be provided with a 5-day notice of any updates or changes in the lists that are implemented in the informatics algorithms.

Genetic Variant Interpretation and Reporting:

A member of the study team, acting as a molecular analyst, will conduct an initial review of the variant data, including review of quality metrics, visual inspection of variants, and review of the literature. The results of the analysis will be presented to the molecular sign-out committee (board-certified clinical geneticists, genetic counselors, clinical molecular geneticists and pathologists) for discussion. Final interpretations will be added as part of that variant's annotation in the database, so that future instances of that variant can be consistently assigned. The research team will review all variants identified as being possibly reportable (see below for detailed procedures). Those judged to be clinically relevant would be confirmed in the CLIA-certified MGL using the duplicate DNA sample.

Indication-based analysis

In the "Diagnosed Cohort," we will perform an "indication-based analysis" that evaluates variants in genes within a specific diagnostic list that is constructed so as to interrogate all known genes that could be related to a patient's phenotype. In the setting of a diagnostic evaluation, we will review all variants in genes that could be related to the phenotype, using a computational classifier to prioritize variants for analysis (Table 1). Since these individuals are already diagnosed with a rare genetic disorder, we will return variants that are deemed to be "pathogenic," "likely pathogenic," or "variant of uncertain significance," according to accepted practice guidelines developed by the ACMG. It should be noted that the computational prioritization is strictly intended as a way to facilitate human review of the data, and will not constitute an automated assessment of variant pathogenicity. For instance, it has been our experience in prior exome-sequencing related studies that many variants that had previously been identified as pathogenic in databases of human mutation have subsequent evidence calling this pathogenicity into question, supporting the need for manual review of variants even in this high-priority class.

Table 1: Computational classification of genomic variants to prioritize for human review

Class	Present in database of human mutations[1]	Variant Type[2]	Minor Allele Frequency (MAF)[6]
A	Yes	Any	<5%
B	N/A	Truncating[3]	<1%
C	N/A	Missense	<1%

D	N/A	Synonymous, Non-canonical splice site[4], and UTR[5]	<1%
E	N/A	Intronic	<1%
F	N/A	Truncating and Missense	1-5%
G	N/A	All other variants	1-5%
H	N/A	Any	>5%

1. Databases to be used in this computational analysis include HGMD and the NCBI ClinVar database. Variants that qualify for category A are those identified as "Disease Mutation" (DM) in the HGMD or variants identified as "Pathogenic" or "Likely Pathogenic" in ClinVar.

2. For the purpose of computational classification, the "variant type" will default to the most damaging effect for the variant among all of the transcripts represented in the RefSeq database. For example, if the variant has a missense effect in one transcript but is intronic or UTR in another transcript, it will be treated as missense for the purposes of computational classification.

3. Truncating variants include: nonsense, frameshifting insertions/deletions, and canonical splice site alterations (the first two and last two nucleotides of the intron).

4. Non-canonical splice site variants include those that occur within 3-10 nucleotides of the intron-exon border.

5. UTR variants are annotated as being located in the 5' untranslated or 3' untranslated regions of the mRNA.

6. MAF data will be derived from frequency data from the 1000 Genomes Project and ExAC; the highest of the minor allele frequencies for a given variant from any ethnic group will be used to evaluate the MAF threshold criteria.

Integral to the efficient diagnostic assessment of an entire genome or exome will be the establishment of *a priori* panels of genes to be assessed under certain clinical situations. One of the major tasks of the clinical and molecular teams will be the development of such lists relevant to the categories of disorders that are present in the Diagnosed Cohort. Once established, these lists will be used to query patients' variant data to identify all variants in genes of possible diagnostic significance in the context of their medical presentation. A molecular analyst will evaluate the prioritized variant list and provide a preliminary interpretation of the case to the molecular sign-out committee.

The committee will make a final pathogenicity determination and decide whether any variants exceed our threshold for reporting (Table 2). Because of the presence of a phenotype in the individual being sequenced, results considered to be clinically relevant would include the "known pathogenic," "likely pathogenic," and "variant of uncertain significance" as determined by the molecular sign-out committee.

Table 2: Categories of findings deemed reportable for an indication-based analysis

Result Category	Variant types	Zygosity	Phenotype[8]	Inheritance
Positive-Definitive	KP[1]	Heterozygous	Concordant	Dominant[9]
	KP	Homozygous or compound heterozygous[6]	Concordant	Recessive[10]
Positive-Probable	LP[2]	Heterozygous	Concordant	Dominant
	KP	Potentially compound heterozygous[7]	Concordant	Recessive

	LP	Homozygous or potentially compound heterozygous	Concordant	Recessive

Uncertain-VUS	VUS[3]	Heterozygous	Concordant	Dominant
	VUS,	Homozygous	Concordant	Recessive
	VUS plus KP/LP[4]	Compound heterozygous or potentially compound heterozygous		
Uncertain-AR het	KP/LP[5]	Heterozygous	Concordant	Recessive
Uncertain-Contributory	Any	Any	Partially matching	Any

1. KP = Known Pathogenic
2. LP = Likely Pathogenic
3. VUS = Variant of Uncertain Clinical Significance
4. In conditions with recessive inheritance, we may identify one KP or LP variant and one VUS. In this situation, we would report the findings as a type of "Uncertain" result due to the presence of one VUS allele. Parental studies would be requested to determine the phase of the variants.
5. In conditions with recessive inheritance in which we only find a single KP or LP variant, but are unable to identify a second candidate variant, we will report this finding as a type of "Uncertain" result due to the possibility of a missed exonic or partial gene deletion on the opposite allele.
6. With NGS technology, it may not be possible to determine the phase of two variants that are identified. When possible, we will use data from the aligned sequence reads to determine phase. If the two variants can be shown to be on opposite strands using NGS data, they will be reported as "Positive-Definitive" with parental studies requested for confirmation of phase.
7. When aligned sequence data are unable to determine the phase of two candidate variants, they will be deemed "potentially compound heterozygous" and reported as a "Positive-Probable" result until parental studies can be performed to determine phase.
8. If the phenotype of the diagnosed individual matches with the condition predicted by the genetic results, this will be considered a "concordant" result. However, if the phenotype of the diagnosed individual only partly matches or is incompletely explained by the genetic results, this will be considered a "partially matching" result. In cases with "partially matching" phenotype, the result will default to an "Uncertain-Contributory" result communicated with appropriate caveats.
9. Dominant inheritance includes both autosomal and X-linked dominant conditions. For X-linked dominant conditions relevant variants would be hemizygous in males and heterozygous in females.
10. Recessive inheritance includes both autosomal and X-linked recessive conditions. For X-linked recessive conditions, relevant variants would be expected to be hemizygous in males and homozygous or compound heterozygous in females.

NGS-NBS and Additional Genomic Findings

In the "Well-Child" cohort there will be no phenotype to inform a diagnostic list. In addition, all conditions unrelated to the phenotype known for patients in the "Diagnosed Cohort" would be considered "incidental" to their primary indication for sequencing. Therefore, the analysis of the NGS-NBS list and additional genomic findings will be more akin to screening than diagnostic testing. In this setting, the prior probability that an individual has a rare Mendelian disorder will be very small (based on the population prevalence of the disorder), and thus the positive predictive value of genomic information will be strongly influenced by the specificity of the results. Both the "Diagnosed Cohort"

and "Well-Child Cohort" will receive results from the "NGS-NBS" gene list. In order to minimize false positives, we will use stringent criteria for return of results and will only return variants that are deemed to be "pathogenic" or "likely pathogenic" and consistent with the expected inheritance pattern of the condition (eg. homozygous or presumed compound heterozygous variants in the case of a recessive condition).

Variants will be computationally selected for their presence in a gene on the NGS-NBS list, their likely pathogenic significance and, in the case of recessive conditions, whether one or two mutations are present (34). Given the large number of possible genomic findings, and the low *a priori* likelihood that individuals in the "Well-Child" cohort would be affected with any given rare genetic disorder, it will be critical to strike a balance between the "sensitivity" and the "specificity" of the analysis so as to correctly identify individuals at high risk for a treatable genetic condition without overwhelming the molecular analysts, MGL, and clinicians with large numbers of variants of uncertain significance. This analysis will utilize the same computational classes as described in Table 1, with additional informatic filtering to determine which variants qualify for human review. We will review variants that satisfy the following conditions:

1) For genes associated with conditions inherited in a dominant fashion, we will review any variants in computational classes A and B;
2) For genes associated with conditions inherited in a recessive fashion, we will review variants in cases where two or more variants from computational classes A, B, or C are present.

Cases fulfilling these criteria will undergo review by a molecular analyst and the molecular sign-out conference, and only variants determined to be "known pathogenic" or "likely pathogenic" and consistent with the expected inheritance pattern would be returned in the context of NGS-NBS. All other cases will be reported as "negative." This approach is an inherently conservative one. We recognize that the strict thresholds outlined above will inherently have imperfect sensitivity for detecting clinically relevant variants, but at the same time this approach will have higher specificity and therefore protect against false positive results. This is also a pragmatic approach, since it will be impossible for a human to comprehensively review each of the many variants that will be identified in every individual. Thus, the balance we are striving to achieve is to maximize clinical sensitivity and specificity, while minimizing the effort required of a human molecular analyst.

CLIA Confirmation:

All results deemed reportable in the NC NEXUS study will be confirmed by orthogonal methods (Sanger sequencing) in the CLIA-certified UNC Hospitals MGL (Mol Gen Custom DNA Sequencing, see Appendix 7). A clinical report will be generated and approved by a board-certified molecular geneticist or pathologist. This result will be provided to the parents and will be eligible to be included in the electronic health record (NC NEXUS NGS-NBS Electronic Medical Record (EMR): Appendix 20 and NC NEXUS Additional Results Electronic Medical Record (EMR) Consent: Appendix 21).

Randomization:

After sequencing and analysis of the indication-based analyses and/or NGS-NBS are complete, the couple will be randomized into a "decision group" or a "control group". The couple will be contacted to arrange a return of results visit with a geneticist and genetic counselor.

Randomization will be computer implemented using permutated block randomization with blocks of randomly varying size. Participants will be stratified for block randomization based on three parameters: study cohort ("Diagnosed Cohort" or "Well- child Cohort"), language preference ("English" or "Spanish"), and the relationship status of the parent(s) giving consent ("Single" or "Couple"). In this way, we will achieve optimum randomization within each of these groups.

Those in the "decision group" will be given information about this visit and future study activities. In addition, they will also be given access to a supplement to the electronic decision aid that explains the three categories of additional genomic findings (adult-onset medically actionable, childhood-onset non-medically actionable, and carrier status), and how such information might be of potential benefit or harm**.** They will be able to decide which categories of additional findings to learn (all, none, or any combination of some of the categories). We will encourage parents to use the aid at home prior to the return of result visit. During the visit, they will have the opportunity to discuss the information about the categories from which they can request results and how to communicate their decisions about requesting categories of additional genomic information. They will be asked to sign a form that documents which categories of results they have requested.

The "control group" will not have the option to request the "additional genomic findings" and will not receive access to the supplement to the electronic decision aid. They will only receive information about the upcoming return of result visit.

Return of Results Visit:

Qualified genetic professionals (physicians and genetic counselors) who are part of the research team will meet with the couples after sequencing is complete to disclose clinically confirmed variants that meet a high bar for evidence of pathogenicity. This form of return of results accompanied by comprehensive genetic counseling is the gold standard in genetic testing, and will be complemented by parental utilization of the decision aid and genetic counseling during the first study visit. Negative results will be accompanied by a discussion of the limitations of NGS- NBS, essentially communicating the caveat that a negative result does not fully rule out the possibility of any health conditions developing in the future, as is the case with any screening test.

In cases where the family cannot return for an in person visit, for example, if they have moved from the area, we will return results by telephone or Skype. The reports will be emailed securely before the call so parents can review it prior to our discussion.

Clinically confirmed results will be summarized by a laboratory report given to the couple at the visit. They will be asked whether or not they consent to having the results placed into the child's medical record and will sign a form indicating this decision. A clinical follow-up plan for all results will be established with the parents.

Relatives, primarily parents of children in the diagnosed cohort, may be asked to provide a saliva sample to help us interpret a variant or combination of variants that may provide an explanation for the child's symptoms. In the case of recessive conditions, like many of those tested for in NC NEXUS, there may be two genetic variants identified but the laboratory test cannot tell whether they are each present in different copies of the gene (inherited separately from each parent) or together in one copy of the gene (both inherited from the same parent). This information can be important in determining whether the variants that were detected fit with a recessive inheritance pattern for the condition. In other cases, the laboratory cannot immediately determine whether a variant is harmful or not. These variants are called "variants of uncertain clinical significance" (VUS). A VUS result means that a genetic variant was found, but there is not enough information about it to know for sure whether it cause disease or not.

An estimated 50 relatives will be consented before a sample is obtained. The sample will be labeled with a NC NEXUS study number. When the testing is completed, the lab will report the results to a certified genetic counselor or medical geneticist on the research team by a secure email and they will report the results to the relative by phone. The child's clinical report will be amended to include the information learned as a result of testing; however, the relative will only be identified by his or her relationship to the participant and not by name. Parents will be counseled that the accuracy of the results depends upon knowing the true biological relationship of the relatives being tested and that if the stated father of a participant is not the true biological father, the interpretation may be incorrect.

Subsequent Return of Additional Results (Decision Group only):

Election to receive any additional results will trigger an independent analysis of genomic data and CLIA confirmation. Couples requesting results from the carrier status category
will be able to learn these results during a scheduled phone call with a genetic counselor.
Requests for the other two categories will trigger a second return of results visit with a geneticist and genetic counselor. Couples in the "decision group" will be asked to complete a brief post-decision questionnaire (the Time 2A questionnaire) after they decide whether or not to request additional genomic findings to assess

the consequences of having this option.

Follow-up Questionnaires:

All participants will complete two follow up questionnaires following the return of result visit; a Time 3 questionnaire completed within 2 weeks of the visit and a Time 4 questionnaire completed 3 months after the visit. Both will assess the short- and longer- term consequences of decision-making and results disclosure.

Couples who do not complete a questionnaire in a timely manner may be offered the option of completing questionnaires in a telephone interview. Questionnaires will be administered in Qualtrics, accessed via a computer or mobile device, with paper and pencil versions of the questionnaires available to those who prefer not to complete them online.

Follow-up procedures

Revised results:

Advancements in medical genetics and our understanding of variant pathogenicity will continuously evolve and thus impact the clinical interpretation of variants identified in the study participants. One scenario that can be anticipated is that the pathogenicity assessment for a particular variant may change over time. This means that the initial classification of a variant may be superseded by a subsequent reclassification. This could mean that a result that was previously "negative" could change to "positive" if a variant initially classified as VUS is reclassified as pathogenic. It is also possible that a variant initially classified as "pathogenic" or "likely pathogenic" would be reclassified to VUS. In this scenario the previous "positive" result could change to "negative." These types of revised result will be communicated to participants without notification of the FDA.

In addition to the expected reclassification of variants, there are other types of revised results that we can anticipate. Over time the association of more genes with diseases and the development of prevention or treatment will result in reassignment of loci and lead to changes in the interpretation of sequencing results. In addition, advancements in sequencing technology, bioinformatics analysis, and variant assessment will inherently necessitate periodic alterations of the established analytic pipelines. Among the major research activities of the NC NEXUS project will be the refinement of the genes/loci that are assigned to each category of genomic information (as defined above), evaluation of new bioinformatics algorithms, and the criteria used to assess pathogenicity of variants.

- Advancements in the science of medicine will continuously add to our knowledge of the genetic underpinnings of disease. Although most of the inborn errors of metabolism that will be present in the "diagnosed cohort" have definitively established genetic etiologies (eg. PKU, MCADD), other conditions represented in this cohort (eg. hearing loss) are still subject to active investigation. Therefore, the

diagnostic lists utilized for the "indication-based analysis" will be updated periodically to include newly discovered genes, when the research team deems those discoveries to have sufficient clinical validity. Similarly, new treatments and management strategies will be defined for many genetic conditions, thus changing their potential clinical actionability. Therefore, the list of genes that constitutes the "NGS-NBS" may be updated to account for such advancements. Finally, newly discovered genetic conditions will be reviewed and assigned to other categories of genomic information (adult-onset medically actionable, childhood-onset non-medically actionable, and carrier status) as appropriate.

- Advancements in computational processing and analysis of NGS data will continuously improve the analytic validity of variant calling pipelines, improving the sensitivity and specificity of the variants that are identified. Therefore, we will utilize the raw sequence data generated through the NC NEXUS project (and other ongoing projects at UNC) to evaluate new informatics pipelines. These analyses will be performed in parallel with the established procedures described above, and only when the research team identifies substantially improved performance will the pipelines described in section 2 be updated.

- Optimizing the criteria for analysis and reporting of genomic variants in the context of NGS-NBS is a core research question for the NC NEXUS project, and thus defining the most effective informatics algorithms is expected to be an ongoing task. For example, one goal of the research project is to investigate informatics algorithms that can be used to select variants for human review, optimizing the clinical sensitivity and clinical specificity while minimizing human workload. Therefore, we will utilize the variant data generated through the NC NEXUS project (and other ongoing projects at UNC) to evaluate these algorithms, in parallel with the procedures described above. One example will be the development of algorithms that can evaluate variant annotations in order to satisfy specific criteria for pathogenicity assessment, in order to provide a more accurate preliminary classification than the computational classifier described in Table 1. The algorithms will be updated only when the research team is satisfied that an updated informatics algorithm is superior.

If, as a result of any of the advancements described in the bullets above, the research team determines the need to update gene lists, bioinformatics pipelines, variant analysis algorithms the FDA will be provided a 5-day notice. All participants analyzed using the previous version of the protocol will have their data reanalyzed, and in the event that any new findings qualify for return of results,

they will be confirmed in the MGL. Parents will be re-contacted that the results they have received during the study have changed as a result of reanalysis.

Thus, by design there will be developmental changes in the protocol, accompanied by periodic reanalysis of the NGS data, with the possibility that the results of the analysis may change over time and some participants will be re-contacted for updated
results. This situation will be clearly described in the informed consent.

Longitudinal follow-up of results:

The downstream implications of positive results are of great interest for the NC NEXUS project. We will therefore plan to engage in longitudinal follow-up for the duration of the study (as long as funding allows). This follow-up will include both clinical follow-up and psychosocial evaluations of parents.

4 Clinical follow-up

The clinical follow-up of participants in the NC NEXUS study will depend on the cohort to which they belong and the type of result they receive.

- ***Previously known diagnoses:*** Participants from the "Diagnosed cohort" will already have established standard-of-care follow-up through the specialty clinics from which they are recruited, and their participation in the study will have no impact on this ongoing clinical care. Results will be provided to the parents by a certified genetic counselor and MD medical geneticist, and communicated to their clinical providers via secure messaging. Any variants that are confirmed in the CLIA lab will be eligible for placement in the EHR. The findings may be utilized to guide care, but this would be entirely at the discretion of the established clinical providers. In this case, clinicians associated with the NC NEXUS study will serve as consultative resources for clinical providers but will not direct the care of the patients. Study personnel will actively monitor the EHR as part of an observational study to track how genomic findings are utilized.

- ***Previously unknown diagnoses:*** Participants in both the "Well-child cohort" and "Diagnosed cohort" will have the potential to receive positive findings from
NGS-NBS or other additional categories of genomic information that will represent new information (i.e. unrelated to a diagnostic indication). These results will be provided to parents by a certified genetic counselor and MD medical geneticist, and a standard-of-care clinical follow-up plan will be established that is appropriate for the finding. This customized plan may involve long-term monitoring by a pediatrician, diagnostic imaging, other screening tests, and referral to specialists. This follow-up plan will be part of the patient's clinical care. Clinicians associated with the NC NEXUS

study will serve as consultative resources for clinical providers but will not be directly responsible for the care of the patients. Study personnel will actively monitor the EHR as part of an observational study to track how genomic findings are utilized. In order to mitigate medical risks in this population, study providers will determine specific benchmarks (depending on the individual finding) that will be evaluated to ensure that appropriate follow-up is being given. Study personnel will track these benchmarks and, if a benchmark is not met, we will communicate with the parent to determine why and to develop an alternative clinical follow-up plan depending on the situation.

- ***Negative results:*** Most participants in the "Well-child cohort" and some participants in the "Diagnosed cohort" will have negative findings. Results will be provided to parents by a certified genetic counselor and MD medical geneticist, parents will be counseled regarding the small chance of a false negative result, and the patients will undergo routine clinical follow-up with their providers. Study personnel will passively monitor for rare cases of false negative genomic results by inviting parents to contact the study in the rare event that symptoms of a genetic disorder develop in their child.

Outcomes will be tracked in every participant by way of a chart review performed at the end of the study period. This review will include developmental outcomes, clinical events specific to any diagnoses, and results of any screening or diagnostic tests.

Indication-based reanalysis:

All parents in the study will be given an opportunity to request an "indication-based analysis" should symptoms of a genetic disorder arise in their child. We anticipate that such requests will be rare. If a request is made, an appropriate diagnostic list will be developed (depending on the child's symptoms) and molecular analysis will be performed in accordance with section 2 above. Results will be confirmed in the CLIA lab and provided in accordance with section 2 above.

Schedule of activities

The schedule of activities is shown in the Study Design Schematic above

The enrollment telephone call will take an estimated 15 minutes to review the parents' participation consent form and schedule Visit 1.

- Visit 1 will take an estimated 30 minutes in order to allow parents to have any questions answered and make a final decision about whether or not to accept NGS-NBS for their child. We expect that this encounter will be facilitated by the parents having access to the electronic decision

aid prior to the visit.

- Visit 2 will occur approximately 3-4 months after Visit 1. For most participants in the "Diagnosed Cohort" the return of results is likely to take 30 minutes to review the indication-based analysis. For >95% of participants in the "Well-Child Cohort" the return of results is likely to take 15 minutes or less for negative results, whereas for the small number that do have a positive finding the return of results could take 30-45 minutes to provide contextualized information and recommend a follow-up plan. For the two-thirds of participants randomized to the "decision group" we estimate that 30 minutes will be needed to review any questions the parents have about the additional categories of genomic information that they may decide whether or not to learn.

- Visit 3 will occur approximately 1-2 months after Visit 2. The length of the visit will depend on how many categories of additional information are requested. We predict that the most typical result will be positive carrier status for 1-4 conditions and negative findings for the other two categories (adult-onset medically actionable, childhood-onset non-medically actionable). For this type of visit we estimate 30 minutes for return of results. In the rare event of a positive finding in the adult-onset medically actionable or childhood-onset non-medically actionable categories we would expect the visit to last 60 minutes or more if needed.

5 Study outcome evaluations

Primary Outcome Measure:

Parental Decision Making Using Decision Aid. [Time Frame: average of 3-6 months.]

Analysis of parents' decisions after they complete an on-line decision aid to see if they wish to participate in the study. Parent participants randomized to the decision group, after their child is sequenced, may decide what, if any, additional categories of conditions they wish to learn about. Options will be yes, no, or unsure.

Sensitivity of Whole Exome Sequencing in Detecting Conditions by Comparing Results of Sequencing to the Appropriate Genes Associated With the Child's Underlying Condition. [Time Frame: approximately 3-6 months after DNA sample obtained.]

Investigators will analyze sequencing results in the diagnosed cohort to determine the ability of whole exome sequencing to detect pathogenic variants in genes related to phenotype.

Secondary Outcome Measures:

Participant Characteristics and Reactions to the Study Will be Collected Through Surveys. [Time Frame: Baseline and 3 months after final return of results visit.]

Surveys are administered at Time 1 (baseline), after parent participants decide to join phase I of the study, which involves agreeing to view the online decision aid about Next-Generation Sequencing Newborn Screening (NGS-NBS); at Time 2, after parent participants have viewed the decision aid and made a decision about accepting NGS-NBS for their child (yes/no/need more information; note that the timing is variable, depending on when participants complete this task, but may range from hours to weeks); at Time 2a (for parent participants randomly assigned to learn/make decisions about additional information), after parent participants have made the decision to accept none, some, or all categories of additional information (note that the timing is variable depending on when parent participants complete this task, but occurs after return of NGS-NBS results); Time 3, which occurs 2 weeks after return of NGS-NBS results; and Time 4, which occurs 3 months after Time 3.

Study endpoints

Primary Objective 1: Scientific endpoints for this objective will be a.) generation of whole exome sequence data and variant call files, b.) analysis of variants to determine whether any disease-causing variants exist, c.) confirmation of any suspected variants in the CLIA laboratory as a measure of analytic validity, and d.) comparison with clinical data to evaluate the sensitivity and specificity of sequencing.

Primary Objective 2: Scientific endpoints for this objective will be a.) curation of gene- disease pairs to define clinical actionability, b.) determination of an actionability threshold for inclusion in the NGS-NBS gene list and definition of the categories of additional genomic information, c.) return of results and observational study of patient outcomes and integration of genetic findings into clinical care.

Primary Objective 3: Scientific endpoints will occur when parents consent to: a.) participate in the NC NEXUS study b.) receive genetic sequencing results (NGS-NBS) for their child c.) if randomized to the "decision group", decide whether to receive results in addition to NGS-NBS. An additional endpoint will occur if clinically significant variants are returned to parents. The final endpoint will take place when parents complete a series of quantitative measures to assess a range of factors related to participation in the study. Please refer to the list of study measure in NC NEXUS Project 3 Longitudinal Study Measures: see Appendix 19).

Sample size determination

This project is one of a consortium of NICHD/NHGRI –funded "NSIGHT" projects. Given that this is an exploratory study, a formal power calculation cannot be performed. We expect to be able to perform joint analyses of data across the consortium to address certain questions that may require larger sample size.

In addition, the study was designed to address the ELSI (Ethical, Legal, and Social Implications) research questions related to the impact of NGS on patients and their families. Using PASS software, we estimated the statistical power for a multiple regression model of mothers' mean scores predicting the decisional conflict scale with two predictor variables (study group [well- and diagnosed-child groups] and race/ethnicity [Black, White, and Hispanic]) and 10 control variables (e.g., demographics, health literacy, trust in medical community) that account for 20% of the variance in scores, assuming a p-value of 0.05. We assumed the sample would be split equally across racial/ethnic groups, consistent with the distribution in our study population. If study group and race/ethnicity account for 2% or more of the variance after controlling for the other variables, we will have statistical power of at least 82%, indicating acceptable power for comparisons by study group and race/ethnicity. We also examined power for detecting differences in decision to screen across study groups. We estimated power for a logistic regression comparing decision to screen between the two study groups, assuming the well-child group has approximately 70% probability of accepting NGS-NBS (based on our Fragile X NBS study) and using a p-value of 0.05. On the basis of these assumptions, we would have 83% power or higher to detect at least a 12% difference in probability of agreeing to screening between the two study groups, which corresponds to an odds ratio of 2.0.

Outcome data and data analysis

Primary Objective 1:

A. Generation of whole exome sequence data: Datasets will include raw FASTQ short read files, aligned BAM files, and VCF variant call files. Variants will be annotated and deposited in a local database as described in section 2.
B. Analysis of variants: Curated clinical significance of individual variants will be stored in the annotated database. Final case-level results (according to Table 2) for each patient will be recorded. Types of mutations that are detected will be characterized in aggregate.
C. CLIA confirmation: Results from NGS will be compared with Sanger results in the CLIA lab to assess the false positive rate (analytic specificity) of NGS.
D. Clinical sensitivity and clinical specificity: Calculations of test sensitivity and specificity will be performed based upon the diagnostic result and the clinical follow-up.

Primary Objective 2:

A. Clinical actionability curation: Curated literature review data for each gene-disease pair will be stored in a REDCap database. Final scores determined by the binning committee will be recorded and analyzed.
B. Definition of categories of genomic information: Based on curation of age of onset and actionability, the binning committee will determine thresholds that define the four categories of genomic information as described in section 2.
C. Observational study of outcomes and integration of findings into clinical

care: Longitudinal outcomes data will be collected in a REDCap database and analyzed.

Primary Objective 3:

Consistent with the primary research questions for this objective, which focus on ethical use of NGS-NBS, we specify the following primary independent variables: Race/ethnicity (non-Hispanic White, Black, Hispanic), health literacy, and child status (diagnosed vs. well). We specify the following primary dependent variables: sequencing- related distress, knowledge about NGS-NBS and (for the "decision group" only) about additional genomic results (continuous variables), decision to accept or decline NGS- NBS and (for the "decision group" only) additional genomic results (dichotomous variables), and decisional conflict (a continuous variable). Primary analyses will focus on these outcomes in mothers. These analyses involve a between-within design and will be analyzed with mixed linear modeling to accommodate nesting (i.e., assessments nested within participants). Models with continuous outcomes will be implemented with SAS PROC MIXED because (a) it can handle nested data; (b) it handles missing data more appropriately than repeated-measures analysis of variance, which uses listwise deletion of missing data; and (c) it provides a wider range of options for modeling the error covariance structure than general linear model procedures, which assume an error structure that is often unrealistic. Models with dichotomous outcomes will be implemented using mixed-effects logistic regression within SAS PROC GENMOD, which shares strengths similar to those offered by PROC MIXED.

6 RISK ANALYSIS

The NC NEXUS research study was launched in response to a request from NIH (NICHD and NHGRI), to perform research studies to investigate next-generation sequencing studies in newborns. Although some ethical guidelines in the past have raised concerns about testing children for adult onset conditions, there are few, if any, studies looking at the outcomes of such testing. In order to satisfy the directives for obtaining additional information about this, we have proposed the NC NEXUS study to provide research data to begin to answer some of these questions.

Parents enrolled in the study will not be exposed to any significant physical or social harms. It is possible that they could experience psychological distress due to making decisions about having sequencing for their child, or deciding about whether to learn certain categories of information. One of the objectives of the NC NEXUS project is to study precisely these types of impacts that might accompany genomic sequencing of newborns. We estimate that 80% of the family units will be couples and 20% will be single parents, so with enrollment of 400 children in the study we expect to enroll 720 parents (400 female, 320 male). The majority will be between 20-40 years of age.

Children and newborns in the study will be exposed to theoretical physical, social, and psychological harms that have been discussed and debated in the medical literature. An additional source of risk relates to positive results (both true positive and false positive) and false negative results. Again, assessment of the magnitude of these potential risks is a major overarching goal of the NC NEXUS project. We estimate that the distribution of 400 children and newborns ages 0-5 enrolled in the study will be roughly 50% female and 50% male.

Anticipated Risks

Potential risks to which the subjects (parents and their children and newborns) will be exposed as a result of their participation in the clinical study can be divided between generic risks that are inherent to human subjects genetic research and risks that are specific and unique to the NC NEXUS project.

The investigators are well aware of guidelines, opinions, and arguments regarding genetic testing in children for adult-onset conditions (2, 6 and 38). Although concern has been raised regarding potential harms (vulnerable child syndrome, genetic discrimination, parental bonding, among others) there has been a dearth of studies that have tracked whether these actually occur. Use of next generation sequencing raises this to a higher level of importance. Additional research in this area has been recommended by stakeholders (48). This is one of the Primary Objectives of this research study that the National Institutes of Health has deemed important to fund.

PHYSICAL RISKS

Discomfort and distress

Risk: Saliva samples will be obtained from infants and children by use of a sponge that will be swabbed along the cheeks and gums. The degree of discomfort is expected to be minimal but, as in any newborn who is disturbed, could cause crying. This sampling procedure was chosen for this study to minimize infant discomfort (compared to venipuncture or heel-sticks). We expect that relatives who are sampled will tolerate this procedure well.

Mitigation: We will obtain the specimens as quickly as possible (estimate 5-10 minutes) by a nurse with extensive experience handling newborn infants. If the newborn cries excessively during the process of sample collection the collection will be stopped. If insufficient sample (e.g. only one) has been obtained then samples from this infant will not be included in the study.

Effectiveness: Highly effective

Complications of medical management

Risk: Infants and children in the study may receive "positive" genetic findings that indicate a need for medical intervention (longitudinal care, screening tests, procedures). In rare cases, such follow-up may lead to unnecessary interventions in the case of false positives, or complications of interventions in both false positives and true positives.
Participants in the "Diagnosed" cohort will all be receiving ongoing standard clinical care in their respective clinics, and the genetic results are not expected to create any additional risk. We expect that <3% of participants will have previously unknown findings from the NGS-NBS screen or the optional additional categories of genomic information (Amendola et al. 2015).

Mitigation: Any children in the study found to have additional findings will be referred for standard of care clinical management.

Adherence to strict and conservative definitions of "pathogenic" and "likely pathogenic" variant classifications, and overall rules for reporting "positive" findings (as described in 2) will maximize specificity and reduce the chance of false positive results.

False positives will also be minimized in some conditions in which confirmatory clinical testing is available (eg. biochemical assays or enzyme testing).

All participants with positive NGS-NBS findings will have a standard-of-care clinical follow-up plan established and will be referred to the appropriate specialists for surveillance or treatment.

Drs. C. Powell and B. Powell are both pediatricians as well as medical geneticists and have experience in appropriate medical follow-up and referral to specialists for children with genetic disorders.

Effectiveness: Moderately effective. Once a participant has embarked on standard-of- care medical follow-up, we cannot further mitigate the risk of complications that may occur.

Failure to diagnose

Risk: Because next-generation sequencing will not achieve 100% sensitivity, there may be participants in the study with false negative results. In addition, the selection of conditions for NGS-NBS will only include a small subset of all genetic disorders. Thus, some participants may not be diagnosed with a condition that is present or will manifest in the future. This is an extremely unlikely outcome for participants in the "Diagnosed Cohort" who have known diagnoses. In addition, due to the very low prevalence of other genetic conditions, it is highly unlikely that any participants in the "Well-Child cohort" will have such a condition. This risk is therefore extremely low.

Mitigation: Participating in the NC NEXUS study does not create any additional risk of a missed diagnosis than any other child in the general population, since they will have equivalent routine standard of care as would any other child in the general population. In addition, elements of the study design will mitigate against this risk:

Continual improvement of bioinformatics pipelines and variant interpretation procedures, with periodic reanalysis, will reduce false negatives by enhancing the sensitivity of the NGS-NBS.

Parents will be offered "indication-based reanalysis" if symptoms develop, thus potentially allowing them to arrive at a diagnosis faster than if they were not participating in the study.

Effectiveness: Highly effective.

Social risks

Confidentiality

Risk: Loss of confidentiality (personal health and genetic information) due to inadvertent disclosure of genetic findings could lead to adverse personal psychological (moderate, rare) or financial impact (e.g. inability to obtain health or life insurance (moderate, rare), or social harm (moderate, rare).

Mitigation: We will apply all reasonable measures to ensure confidentiality for research subjects.

Signed consent forms will be stored in a locked office.
Samples (saliva, isolated DNA, sequencing library samples) and *in silico* data (raw sequencing data, alignment files, called variants) used in this study will be identified using a unique study ID number.
A password-protected secure REDCap database managed by NC TraCS will contain the link between the patient identity and the study ID number, but the research laboratory will not have access to patient identifiers.
Subject identifying information will be accessed only by study personnel with a "need to know" identifying information for the purpose of implementing the study.
In the clinical laboratory, identifying information will be protected in the same manner as all other clinical samples maintained there. Final genetic test reports (which do include the participant's identifying information) are handled via the UNC Hospitals Molecular Diagnostic laboratory according to CLIA standards. Digital copies of the final reports will be password protected and paper copies will be stored in locked cabinets in a secure office space.

Any genetic test results that are entered into the electronic medical record after parental consent will have the same HIPAA protections as any other

medical information.

Participants' responses to questionnaires will be entered into our secure database and identified only by their ID number.

Samples of relatives will be labeled using an assigned an NCX number related to the child's ID number (e.g. NCX_00010-1). The samples will be sent to the Molecular Genetics Laboratory for site-specific testing.

Following testing, the results will be conveyed to the genetic counselor/medical geneticist by secure email and he or she will communicate the results to the relative(s). The child's clinical report will be amended and it will state if there has been a change in the interpretation of the results in light of the additional testing.

Relatives may be identified by their relationship to the child (child's parents) but not by their name or other identifying information. Relatives will not be issued an individual report. In most cases, the additional testing will be of the parents and the medical geneticist/genetic counselor will discuss the option of testing with them at the time of the return of the child's results.

In the rare case that a sample from relative who is not the parent would be useful, the genetic counselor/medical geneticist will ask the parent to contact the relative and have the relative contact the study office to schedule a study visit at the CTRC. Consent will be obtained from the relative by the genetic counselor/medical geneticist and the sample obtained and processed as described above.

Effectiveness: Highly effective

Financial

Risk: There is always a risk that genetic findings could result in financial risk such as loss of insurance or employment, however that risk is very low, both in general and in the current study. Some participants will receive genetic test results that may diagnose a particular condition or indicate that the participant is at-risk to develop a condition in the future.

For participants in the "Diagnosed cohort," the risks are not greater than they would be if the participant were having clinical testing. The risk is somewhat greater in sum because more genes are being analyzed and because of the small chance of an additional genomic finding with significant clinical implications.

For participants in the "Well-Child" cohort there is a very small chance

(<3%) of a finding in the category of "NGS-NBS" results. However, since these findings will be clearly actionable from a medical standpoint, there would be a significant medical benefit to the subject any time such a finding is revealed.

For participants randomized to the "decision group" of the study, the risks of disclosing information about additional genomic findings are not known.

Mitigation: Federal legislation called the Genetic Information Nondiscrimination Act (GINA) prohibits the use of genetic information to discriminate against individuals in employment and health insurance settings. The informed consent process, as well as, the consent forms (see NC NEXUS_consent_phase II_diagnosed cohort: Appendix 16 and NC NEXUS_consent_phase II_Well-Child cohort: Appendix 17) will include a discussion of the benefits and the limitations of GINA. There are also North Carolina state laws to protect against genetic discrimination. A major provision of The Affordable Care Act (ACA) of 2010 prohibits issuers of health insurance from discriminating against patients with genetic diseases by refusing coverage because of 'pre-existing conditions'. Parents will be informed that current laws that protect against genetic discrimination do not apply to life insurance, disability insurance or long-term care insurance (NC NEXUS_consent_phase II_diagnosed cohort: Appendix 16 and NC NEXUS_consent_phase II_Well-Child cohort: Appendix 17). Extensive genetic counseling will be provided to parents regarding their significance of any findings and recommended clinical follow-up. Clinically serious adult-onset genomic findings for which there are no available treatments or preventive strategies (for example, early onset dementia) will not be returned to parents.

Effectiveness: Uncertain. GINA does not protect some "optional" forms of insurance, such as disability, life or long-term care insurance, so there is the potential that participation in this study could affect participants' future insurability for these insurance types. In addition, the law does not apply to the U.S. military. Like GINA, the ACA does not apply to non-health insurance types.

Group Harm

Risk: Because we are including ethnic minorities there is a chance that some genetic findings might be reported as linked to a particular racial or ethnic group, producing what has been described as "group harm." The likelihood of this is estimated to be rare.

Mitigation: We, as clinicians and researchers, are sensitive to this issue and will endeavor to avoid such issues when reporting genetic findings of the study.

Effectiveness: Highly effective

Family Dynamics

Risk: As with any genetic information, there is a possible risk that family members may respond negatively to genetic information that was learned during the course of the research project. This is true of any genetic testing (both clinical and research).

Mitigation: Professional genetic counseling will be provided as part of the informed consent and return of results.

Effectiveness: Moderately effective

Psychological risks

Parental emotional stress due to study participation

Risk: There is a slight risk of parents experiencing uncomfortable emotional states by completing the psychosocial assessments that ask some personal questions about quality of life and experiences of receiving diagnostic results or incidental findings. The degree of this risk is estimated to be rare to infrequent.

Mitigation: Because study interviews and questionnaires were chosen to reflect what are likely to be preexisting concerns, the study assessments are not expected to markedly increase participants' psychological distress. The project team also has had extensive experience in conducting assessments with individuals and families who receive genetic testing and findings from those tests. Before beginning the assessments and interviews, subjects will be reminded that they can stop the interview at any time, or choose not to answer specific questions.

Effectiveness: Highly effective

Parental distress or anxiety regarding positive genomic findings

Risk: The chief risk to parents participating in this study is anxiety or distress from having learned of genomic information about their child that predicts disease risk or reveals a predisposition to a disorder for which there is no currently effective intervention. For parents of participants in the "Diagnosed cohort," the risk of any additional distress or anxiety as a result of study participation is minimal. Parental distress or anxiety is more likely when unexpected genomic findings are returned.

It is possible that family members may respond negatively to genetic information that was learned during the course of the research project. This is true of any genetic testing (both clinical and research) and we expect that professional genetic counseling provided as part of the return of results will mitigate the risk of such outcomes.

Diagnostic Findings: The return of results related to a known diagnosis is straightforward and non-controversial. Emotional distress is possible any time that parents learn their child has a genetic condition. However, parents participating in the "Diagnosed cohort" already know that their child has a disorder with a likely genetic etiology, and research indicates that risk is minimal when genetic information is relayed by a genetic counseling team in an appropriate setting.

NGS-NBS Findings: For parents of participants with positive NGS-NBS findings (not related to a known diagnosis), there is risk for an adverse psychological impact. We expect that the degree of anxiety and distress would be equivalent to that of parents whose child receives a positive standard newborn screen result. This psychological reaction is likely to be tempered by the medically actionable nature of the findings.

Additional genomic findings: For parents randomized to the "decision group" who choose to learn about carrier status in their child, we expect that the psychological impact will be minimal, and similar to that of parents who learn that their child is a carrier of Cystic Fibrosis or Sickle Cell anemia through the standard newborn screening program. For those who choose to learn additional diagnostic information about their child and subsequently receive unexpected information indicating their child has a genetic health risk, the magnitude of psychological distress is unpredictable and depends on the parent and the findings. However, the chance of such findings is very small.

Mitigation: The risks associated with parental responses to genetic information about their child are complex and form the basis of the need for this research project. We will provide genetic counseling and referral to specialists as needed for any positive results.

All study team members who have contact with study participants are already trained (physicians and genetic counselors) to recognize and probe indicators of possible distress (e.g., participants' description or display of distress-related symptoms).

We will collect data on distress (depressive symptoms, anxiety) before return of results (to establish baseline levels) and after return of results, allowing us to examine changes in distress over time.

The decision aid is being designed to help families understand the risks and benefits of study participation and make an informed choice based on their values and preferences.

Parents are also able to change their minds about any choices made as part of the study before information is returned to them.

Effectiveness: Moderately effective

Parental decision regret

Risk: Parents in the study may experience regret about the decision they make with regard to having their child sequenced, or their choices to receive or not to receive certain categories of additional genomic information. Our experience in the clinical setting indicates that emotional distress requiring referrals is rare or infrequent. Our experience in the clinical setting indicates that emotional distress requiring referrals is rare or infrequent. In an earlier study (18) there were no significant differences between 18 mothers of screen-positive infants with Fragile X premutation and 18 comparison mothers on measures of anxiety, depression, stress, or quality of life. A subset of mothers experienced clinically significant anxiety and decision regret, but factors associated with these outcomes could not be identified. Greater spousal support was generally associated with more positive outcomes.

Mitigation: Decision regret will be measured after return of results. Scores will be analyzed to detect clinically meaningful increases (0.5 standard deviations or more, according to research on clinically meaningful changes and changes that are noticeable to research participants).

The decision aid should minimize regret by enabling informed choices based on the parents' values and preferences.

Any participants flagged based on these monitoring methods will be discussed with the study team, which includes clinical geneticists, certified genetic counselors, and psychological researchers with expertise in psychological distress.

For any parents who do experience distress, research suggests it would likely involve anxiety and decision regret rather than depressive symptoms or poor quality of life, suggesting that additional counseling would be a first-line response to resolve the distress, psychological counseling or similar referrals may be indicated.

Effectiveness: Highly effective

Psychological impact on child/infant participants

Risk: Return of unexpected results in the context of testing a minor raises new and challenging issues regarding the protection of human subjects. These are mostly theoretical risks, without a great deal of empiric data to indicate the magnitude or likelihood of these risks. As such, one of the key goals of the NC NEXUS project is to begin to provide evidence on the psychological impact of genetic testing in children. The most common type of "unexpected" result (unrelated to a participant's diagnosis) in this study will be carrier status for a recessive disorder, which we expect to have a very low chance of having a detrimental psychological impact. Other potentially more concerning findings that would indicate the likely future onset of disease will be much less likely, estimated at <3% of the cohort.

Possible psychological risks include:

Vulnerable child syndrome in which genetic findings exacerbate childhood developmental or adjustment problems

Abandonment or neglect of the child as a result of genetic findings

Abrogation of the future "right not to know" genetic information

Mitigation: The NC NEXUS study follows the model of informed, shared decision making by providing educational resources (understandable decision aids paired with counseling) to help ensure, using rigorous practices, that parents are adequately prepared for this information. If such issues arise as a result of learning an unexpected genomic finding, we have extensive local experience and resources to help in such situations.

Dr. C. Powell runs a pre-symptomatic Huntington disease testing program and has experience in such situations. Dr. Berg, Dr. B. Powell and Ms. Roche have had extensive experience returning diagnostic, medically actionable and additional findings in both a clinical and research setting.

Families experiencing significant distress as a result of unexpected findings will be referred to appropriate mental health specialists as needed. Since clinical geneticists and certified genetic counselors will be on the team conveying these results, our experience in the clinical setting indicates that such emotional distress requiring referrals is rare.

Parents will not be able to request results that would indicate a risk of an adult-onset condition for which there is no current treatment, such as ALS.

Effectiveness: Uncertain. Some potential harms, such as the long-term implications of learning about genomic information in a healthy newborn, are unknown and somewhat unpredictable, and may manifest long after completion of the study. Although we plan to follow participants longitudinally as long as possible, we cannot guarantee that funding will exist for long-term uninterrupted monitoring of outcomes decades from now.

7. ADVERSE EVENT RECORDING/REPORTING

The study team has considerable expertise in conducting assessments with individuals and families who receive genetic testing and findings from those tests. Key personnel in this grant include certified genetic counselors, clinical geneticists, medical biochemical geneticists and a neurologist, all of whom have extensive experience with medical management of rare genetic conditions and dealing with patient responses to genetic information including newborn screening results in a clinical setting.

- ***Adverse Event Definitions***

The NC NEXUS study itself does not raise substantial risks for any of the following adverse events:

- Serious injury or illness
- Hospitalization
- Disability
- Life-threatening adverse effect

8. *Medical management of known diagnoses:*

Participants in the "Diagnosed Cohort" will have ongoing medical care for their known conditions, some of which include the potential for serious illnesses, hospitalizations, or even death. However, enrollment in the NC NEXUS research project will not constitute any increased risk for such complications, whether or not a molecular diagnosis is obtained. Participants in the "Well-Child Cohort" will likewise have the potential to develop any typical injury or pediatric illness. Again, enrollment in the NC NEXUS research project will not constitute any increased risk for these common conditions. Therefore, we will ***not*** consider intercurrent illnesses, sporadic injuries, or the worsening of existing conditions as adverse events associated with the study.

- ***Medical management of newly identified diagnoses:***

Positive findings from the NC NEXUS study may lead to clinical follow-up and possibly medical interventions as part of the clinical management of a newly diagnosed genetic condition. These outcomes are expected and will be documented as part of the longitudinal follow-up of participants with positive findings. Therefore, we will ***not*** consider the existence of additional treatment or further diagnostic tests as an adverse event. However, there may be instances in which physicians take actions due to a genetic finding, but these actions are not considered to be standard of care. In addition, there may be rare instances in which the standard of care management of a diagnosed genetic condition leads to complications that would negatively impact the clinical utility of the genetic result. Therefore, we will monitor for any such adverse events, defined as:

- ***Adverse effects associated with the investigational device due to medical interventions undertaken as a result of positive findings:***

1. There is a reasonable possibility that erroneous (non-standard of care) medical actions may have occurred as a consequence of positive findings.
2. There is a reasonable possibility that serious injury, hospitalization, or death may have occurred as a complication of standard of care medical follow-up of positive findings.

- ***Psychosocial complications of newly identified diagnoses*:**

Positive findings from the NC NEXUS study may lead to low-level parental anxiety and/or distress. This is an expected reaction to a medical diagnosis and should be short- lived and relatively minor. Therefore, we will ***not*** consider the existence of mild or time- limited psychological complications as an adverse event. However there may be rare instances in which study members deem the level of psychological distress to require referral to a specialist or other intervention. Therefore, we will monitor for any such adverse events, defined as:

Adverse psychosocial effects associated with the investigational device due to revelation of positive findings: There is a reasonable possibility that serious psychosocial harms may have occurred as a consequence of reporting positive genomic findings to the parents.

- ***False negative results:***

The NC NEXUS study is evaluating a screening test using a technology that is certain to have imperfect sensitivity for most genetic conditions. Therefore, there is a chance that some individuals in the study will develop a genetic condition that was not detected by the sequencing test (false negative results). This is a predictable event, and thus will ***not*** be considered an adverse effect, since these individuals would have the same outcome as if they had not enrolled in the study. With longitudinal follow-up over the course of the study it is possible that we will identify a small number of false negative results, but based on the sample size this is an extremely unlikely occurrence. Furthermore, all participants will already be receiving standard of care newborn screening and pediatric care and thus will not be relying on NC NEXUS for the detection of conditions that are currently deemed to be part of the recommended uniform screening panel.

- ***False positive results:***

NGS technology (like any test) is known to have technical false positives, essentially variant calls that are due to errors in mapping, other variant calling artifacts, or inherent limitations in the current state of knowledge about the human genome (eg. unmapped pseudogenes). All results will be confirmed by Sanger sequencing in the CLIA-certified MGL, so it is very unlikely that any technical false positives will be inadvertently returned to participants. On the other hand, the process of variant analysis and interpretation is part of the practice of medicine and is subject to variability between laboratories. A board-certified molecular geneticist or pathologist will review and sign- out all results that are deemed returnable in the NC NEXUS study in order to ensure the highest threshold of quality. However, there is a possibility that some of the positive results could be reinterpreted in the future (and thus become false positive results). In this case, it is possible that actions may be taken due to the finding, which are later determined to have been unnecessary. Therefore, we will monitor for such adverse events, defined as:

Unnecessary medical care associated with the investigational device

due to false positive findings: There is a reasonable possibility that medical actions taken by the patient's physician were related to a genetic finding that was later deemed to be a false positive result.

- ***Other unexpected adverse effects:***

The NC NEXUS study may also involve risk for unexpected adverse effects, which we define as any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Recording and Assessment of Adverse Effects

All observed or volunteered adverse effects, regardless of cohort or randomization group, that have a reasonable possibility of a causal relationship to the investigational device will be recorded in the REDCap database entry for the participant. For all adverse effects, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the effect (i.e., whether the effect should be classified as a serious adverse effect) and; 2) an assessment of the causal relationship between the adverse effect and the investigational device or, if applicable, the other subsequent treatment or diagnostic procedure.

The minimum initial information to be captured in the subject's REDCap form concerning the adverse device effect includes:

- A narrative description of the event
- Classification of the event's severity and rationale for classification
- Investigator assessment of the association between the event and study treatment
- Current status

Reporting adverse effects to FDA

- **Adverse Device Effects**

The NC NEXUS study itself does not raise substantial risks for any of the

following adverse device events:

- Results in death
- Is life-threatening
- Results in permanent impairment of a body function or permanent damage to body structure
- Necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure

• ***-or-***

- A previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting noted above (reporting such events within 10 working days from when event was deemed reportable).

  Such reports will be submitted within ***10***

working days Unanticipated Adverse Device Effects

(UADEs)
The NC NEXUS does not contemplate having UADEs associated with this study.

• Withdrawal of IRB approval

The Sponsor shall notify the FDA, all participating IRBs and participating investigators of any withdrawal of approval of the study by a reviewing IRB ***within 5 working days*** after receipt of the withdrawal of approval.

• FDA Reporting Process

Medical Device Reports, whether for anticipated or unanticipated device-related effects, are to be submitted on FDA Form 3500A. The contact information for submitting MDR reports is noted below:

Food and Drug Administration
Center for Devices and Radiological Health
Medical Device Reporting
PO Box 3002
Rockville, MD 20847-3003

Reporting adverse effects to the responsible IRB

Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The following describes the UNC-CH IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record.

• *Report Promptly, but no later than 5 working days:*

Researchers are required to submit reports of the following problems promptly but no later than10 working days from the time the investigator becomes aware of the event:

- ***Unanticipated problems including adverse events*** **that are unexpected and related**
  - *Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB- approved informed consent document and other relevant sources of information, such as product labeling and package inserts.*
  - *Related to the research procedures: An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.*
  - *Harmful: either caused harm to subjects or others, or placed them at increased risk*

- ***Unanticipated adverse device effect***: Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

• *Other Reportable events:*

The following events also require prompt reporting to the IRB, though no later than 10 working days:

- ***Complaint of a research subject*** when the complaint indicates unexpected risks, or the complaint cannot be resolved by the research team.
- ***Protocol deviations or violations*** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
  - *one or more participants were placed at increased risk of harm*
  - *the event has the potential to occur again*
  - *the deviation was necessary to protect a subject from immediate harm*
- **Breach of confidentiality**

Reporting Process

The reportable events noted above will be reported to the IRB using the form: "Reportable Event Form" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow- up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

Withdrawal of subjects from the study

The informed consent materials will clearly state that parents can withdraw from the study at any time, that this will not impact the child's medical care in any way. Parents who refuse to use the decision aids or fail to comply with the questionnaires will be considered withdrawn and their child's sample will not undergo further sequencing or analysis. They will not participate in the randomization. Specimens from parents who desire to terminate participation will be destroyed and no further analysis of data in such individuals will be pursued.

Description of Investigational Device

On August 27, 2014, the FDA provided a response to a pre-submission inquiry and determined that the proposed NC NEXUS study would be required to submit an Investigational Device Exemption (IDE) due to the significant risk associated with the potential long-term consequences to both the parent(s) (i.e., psychological impact) and the child (i.e., denial of life and/or long-disability insurance and having medical records containing WES information of which he/she did not consent to) after learning of the WES finding. The FDA has determined that the decision aid that will be developed and the psychosocial research that will be conducted are also part of the "device" that is being evaluated. In that case, the "device" would be best described as:

> "Informed parental decision-making aided by an electronic decision aid regarding their acceptance of next-generation sequencing newborn screening for their child; whole exome sequencing with targeted analysis and Sanger confirmation of positive findings; return of results through standard-of-care genetic counseling; and follow-up questionnaires regarding the psychosocial impact of the screening."

This device encompasses all aspects of our study, including informed consent forms, shared-decision making tools (i.e., electronic decision aid), sample collection, next- generation sequencing, bioinformatics pipelines including variant calling and selection algorithms, confirmation of variants with Sanger sequencing, procedures for returning different categories of genomic results to parents, and follow-up procedures with parents before and after learning of research results (questionnaires).

Each of the steps involved in the NCNEXUS project can be envisioned as an element of an instructional manual containing (but not limited to) the following instructions of use: script used for the initial recruitment, study brochure, decision aids, questionnaires, consent forms, sample collection, laboratory methods (DNA extraction, exome library preparation, and massively parallel sequencing), bioinformatics pipeline (initial informatics analysis and variant annotation), clinical interpretation of exome sequence variants (screening and indication-based analysis), variant confirmation by Sanger sequencing (in the CLIA-certified Molecular Genetics Laboratory), randomization, return of results, and other detailed procedures describing precautions and safeguards that will be utilized before and after the parents have been informed of the investigational results. Thus, the "device" is all aspects of the study as described above.

Possible modifications to the device that we can anticipate occurring throughout the study are discussed in previous sections and briefly summarized here. The clinical actionability of all Mendelian disorders will continue to evolve over the foreseeable future. The development of the list of conditions that have been scored by the NC NEXUS Actionability team (Appendix 8) as described in Section 2, Defining Clinical Actionability is expected to continue throughout the study period and after, and therefore, periodic updates of the lists will occur. Slight changes or adjustments based on feedback from user testing may lead to minor changes in the Longitudinal Study Measures (Appendix 19) described in Section 5, Study Narrative. Among the major research activities of the NC NEXUS project will be the evaluation of new bioinformatics algorithms; in addition, we anticipate improvement in the evidence available to assess pathogenicity of variants (as defined in Section 2 Revised Results in the Follow- up Procedures, Section). Thus, by design there will be developmental changes in the protocol, accompanied by periodic reanalysis of the NGS data, with the possibility that the results of the analysis may change over time and some participants will be re- contacted for updated results. This situation will be clearly described in the informed consent.

The FDA will be provided with a 5-day notice of any updates or changes in the measures, lists that are implemented in the informatics algorithms, or types of revised results that are returned to patients.

Monitoring Plan/Procedures

The Sponsor-investigator (Principal Investigator) and key study personnel will meet on a quarterly basis to discuss aspects of the study, review unanticipated problems and adverse events, evaluate results, scrutinize data and anticipate problems relevant to subject safety. An independent Study Data Monitor and Medical Safety Monitor will be established prior to enrollment of participants. These individuals will meet with the Principal Investigator and other key study personnel as described below.

As our study involves a unique aspect of genomic sequencing in the pediatric population not typically considered under most "medical monitoring" plans,

including the option for some parents to receive results on variants in genes that are associated with carrier status and adult-onset conditions in their infant, we held a 2-day conference at the beginning of our project to solicit opinions about our proposed study protocol including return of results from an external group of consultants including experts in biomedical ethics, genetic counseling, newborn screening and clinical genetics. We received support for our plan as outlined above. These experts have agreed to continue to serve as external consultants throughout our study period and include Dr. Eric Juengst, Director of the UNC Center for Bioethics, as well as others from outside our institution. They will be available as needed to review any ethical concerns or questions that arise.

STUDY DATA MONITOR

The data in this study will be reviewed on a regular basis by an independent data monitor. The role of the data monitor will be to review study documentation, regulatory files, and informed consent to ensure the quality and integrity of the data collected and adherence to good clinical practices. An important focus of the data monitoring will be to ensure appropriate informed consent has been obtained from the research participants.

MEDICAL SAFETY MONITORING

The Principal Investigator will oversee the safety of the study at her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. Medical monitoring will include a regular assessment of the number and type of adverse events as defined in section 8. Any safety concerns or unanticipated problems will be relayed to an independent medical safety monitor for immediate review. In addition, there will be a quarterly project meeting at which concerns can be raised for discussion by the entire project team. Such opportunities will allow us to monitor for the expected psychosocial impact of genetic testing as well as being alert to otherwise unexpected participant safety issues. The medical safety monitor will have expertise in pediatrics and research ethics. The role of the medical officer will be to advise the Principal Investigator and project team and make recommendations about continuing the study.

AUDITING AND INSPECTING

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study-related documents and study-related files. The investigator will help coordinate inspections of applicable study-related facilities.
Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

ADDITIONAL RECORDS AND REPORTS

Reports from the study data monitor describing records reviewed, any concerns

noted, and recommendations made to correct deficiencies; as well as correspondence from the medical safety monitor following any necessary reviews will be provided to the IRB, FDA, and NHGRI/NICHD. Reporting will also include annual progress reports to NHGRI/NICHD.

DATA HANDLING AND RECORD KEEPING

Most of the data collected in the NC NEXUS study will be stored electronically as follows (and detailed below):

- A REDCap database managed by the North Carolina Translational and Clinical Sciences (NC TraCS) Institute will be used to store demographic information and baseline clinical data for each participant (parents and children) enrolled in the clinical study. This database is within the UNC Hospitals firewall and will serve as the database of record linking personally identifiable information with the unique study identifier. Entry and maintenance of the study records will be a shared responsibility of study investigators.
- Project-related tasks and laboratory data will be recorded in a custom workflow management system managed by RENCI that has access restricted to specific roles. No personally identifiable health information is included.
- Data from questionnaires will be stored on a secure drive at UNC with restricted access to only those personnel who are involved in data analysis.
- Data from the decision aid will be stored on a secure drive at RTI with restricted access to only those personnel who are involved in data analysis.
- Sequence data and called variants will be stored in the UNC Research Computing system. Raw sequence data will be stored for the duration of the study on tape backup through UNC Research Computing. A reduced representation of the participant's variant calls (currently in the form of a VCF file, although standard formats may change over time) and a file that comprises the clinically relevant variants to be reviewed and confirmed will be stored in a data repository managed by RENCI and will have access restricted to a subset of study personnel who are involved in data analysis.

Results that are confirmed in the MGL will be reported as clinical genetic test results and parents will be provided with a paper copy of the report for their personal records. These official reports will have participant names and medical record numbers and, if consent is given, will become part of the permanent medical record, subject to the protections afforded by the HIPAA regulations. Otherwise, subject names or other directly identifiable information will not appear on any reports, publications, or other disclosures of clinical study outcomes.

Record Maintenance and Retention

The investigator-sponsor will maintain records in accordance with Good Clinical Practice guidelines, to include:

- FDA correspondence related to the IDE application and Investigational Plan
  - IRB correspondence related to the clinical protocol, current and past versions of the IRB-approved clinical protocol and corresponding IRB-approved consent forms
- Signed Investigator's Agreements and Certifications of Financial Interests of Clinical Investigators
- Certificates of required training (e.g., human subject protections, Good Clinical Practice, etc.) for investigator-sponsor and listed sub-investigators
- Signed informed consent forms
- Copies of adverse event reports and annual or interim reports
- Monitoring visit reports
- Final clinical study report.

Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

All clinical information will be kept confidential, and will only be accessed by those directly involved in the research. The FDA may request and be granted access to the records. The digital file containing the linked participant names, UNC medical record numbers and unique study identifiers will be stored in a password-protected REDCap database managed by NC TraCS. Paper copies of consent forms and any personal health information will be stored in a locked filing cabinet in a locked office. All identifiers (name, date of birth, etc.) will be removed from the saliva samples before they are sent to the BSP or MGL and all samples used in this study will be labeled only with the participant's unique study identifier.

Genetic variant data: Each participant's genetic variant data will be stored using a unique participant ID number and stored for the duration of the study on tape

backup through UNC Research Computing. A file that comprises the clinically relevant variants to be reviewed and confirmed will be stored in a data repository managed by RENCI, which will have access restricted to a subset of study personnel. A reduced representation of the participant's variant calls will be stored with the unique participant ID number to allow for re-analysis.

Questionnaire Responses: Participants' responses to study questionnaires (research data) will be identified only by their unique participant ID number, whether collected in an online questionnaire format implemented in Qualtrics or in a paper-and-pencil questionnaire or interview by a trained staff member (e.g., for participants who cannot or prefer not to complete the online questionnaire). The questionnaires will not collect information that could be used to identify participants.

Decision Aid Usage Data: Data gathered through participant interaction with the electronic decision aid (e.g., app performance metrics, usage metrics, and participant inputs) will be associated with unique participant ID numbers. The decision aid will not collect information that could be used to identify participants. All usage data will be stored in a secure password protected database at RTI secured by industry standard firewalls and a stringent IT security policy framework. Data and query tools published via web interfaces will be encrypted.