Resident Scholarly Project / Resident Proposal
THIS PROJECT IS UNDER REVIEW BY THE IRB. THIS IS A PRELIMINARY PROPOSAL.

Name: Joshua Motelow, MD, PhD Date: 7/22/2016

Faculty Mentor: David Goldstein, PhD

Title of Project: Understanding the utility of whole exome sequencing in the NICU

1. Study Purpose and Rationale

Mendelian or monogenic diseases are caused by mutations in a single gene. There are more than 7,000 known Mendelian disorders of which ~4,000 have a known molecular basis.[1-3] ~85% of mutations that cause disease occur in the exome.[4] They are rare although genetic disorders are though to occur in up to 80 per 1000 live births.[2] Genetic causes make up 20% of all infant mortality in the United States.[5] While not always treatable, the knowledge of diagnosis may provide comfort to many families.[6]

Whole exome sequencing (WES) is an emerging technique in identifying diagnoses in rare disorders. WES utility has been studied in select patient populations. Of 250 consecutively referred for WES, 25% achieved a molecular diagnosis.[7] 80% of these patients were referred with a primarily neurologic phenotype. In patients referred for epilepsy, ~38% achieved a diagnosis while patients with epileptic encephalopathy were diagnosed at a rate of ~43%.[8] In another large study observational study published in JAMA, an overall molecular diagnosis was reported in ~25% of patients.[9] In this study, 4% of patients had medically actionable diagnoses. WES can often correct previously assumed diagnoses. In a select population of patients whose parents were consanguineous, 8% had a previous diagnosis corrected or modified by WES.[10] "Trio" analysis in which the exomes of both parents are sequenced can improve yield on WES analysis up to ~37%.[11,12]

But what about its use as a tool for diagnostic screening? Early studies indicated that 25% of Mendelian disorders are apparent at birth.[13] Neurodevelopmental disorders affect ~5% of the population whose care may account for up to 10% of health spending.[14] A diagnosis with WES has the potential for circumventing the "diagnostic odyssey."[15,16] WES studies have been primarily limited to populations that are typically tested by traditional genetic testing. The diagnostic tests for identifying these diseases may include imaging, biopsy, and metabolic testing. Traditional genetic testing includes karyotype, chromosomal microarray analysis, fluorescence in situ hybridization, single-gene tests, gene-panel testing, specialized genetic biochemical laboratory tests (such as urine organic acid analyses, acylcarnitine profile, and plasma amino acids), and studies for the mitochondrial genome. Costs may range from $10,000 to greater than $25,000.[16,17]

An admission to the neonatal intensive care unit (NICU) is an upsetting event for parents and clinicians. A small study of acutely ill infants examined the utility of rapid sequencing tests in in this population.[18,19] 32 children had both traditional genetic testing (array comparative genomic hybridization, fluorescence in-situ hybridization, high-resolution analysis of chromosomes, sequencing of genes and gene panels, methylation studies, and gene deletion or duplication assays) and whole genome sequencing (WGS). Standard testing provided diagnosed 3 (9%) of patients. WGS diagnosed 2 of these infants, missed 1, and also provided a diagnosis in 18 additional infants (57%). Management changed in 13 of the 20 patients diagnosed with WGS due to the WGS result.

This project aims to understand the impact of a large scale implementation of WES in a level III NICU. Specific outcomes include changes in clinical management and length of stay although the qualitative benefits and drawbacks of such a global program will also be assessed. We are optimistic that such a program will provide clinicians data to improve patient care and families the certainty of diagnosis.

2. Study Design and Statistical Procedures

This is a quality improvement study and may engage in plan-do-study-act (PDSA) cycles

The primary analysis will be the frequency that WES changed clinical management or affected length of stay vs. traditional genetic testing for which Fisher's exact test will be used. Length of stay (LOS) will be assessed by Wilcoxon rank sum test. Logistic regression may be used to determine if there are differences between groups such as gestational age, respiratory support, or positive microbiology cultures.[8] Statistical considerations include censored data (death prior to discharge), effect of WES on type of traditional genetic testing ordered, role of WES in those patients who are not critically ill but still undergoing genetic testing, changes in NICU practices from 2015 to 2016 (matched controls based on admitting diagnosis?), and comfort care decisions.

Smaller studies of high-risk infants suggest a diagnostic yield of 9% from traditional testing and 57% of WGS. [18] Given these numbers, only 18 infants in each group will be necessary to find a difference between the two groups with respect to diagnosis. In a study by Dr. Goldstein's group of the yield of traditional diagnostic testing, 46% of sequential patients presenting an outpatient genetics clinic received a genetic diagnosis.[16] Assuming 1000 patients in the NICU and assuming genetic testing in 100, WES would have to exceed a 66% diagnosis rate.

3. Study Procedures

The NICU at CHONY cares for > 1,000 neonates each year (30% transfers). 60% of newborns in our NICU are premature. Once clinicians have ruled out maternal factors

as causes of the NICU admission, families will be offered WES as part of a research study.

Sequenced data is aligned to reference genomes and loci from the proband that differ from the genome will be identified as “variants”. These variants are subsequently run through in house software (ATAV) to assess their potential for pathogenicity using both external (e.g. ExAC) and internal controls (>12,000 exomes and 1000 whole genomes). Potentially pathogenic variants will be assessed for their significance with functional assays. WES analysis will be completed in 4 weeks with positive results requiring additional testing in results will be made available in approximately four weeks from receipt of the trio samples in the laboratory.

4. Study Drugs or Devices

n/a

5. Study Instruments (e.g., Questionnaires, Interview Outlines, Focus Group Guides)

Blood will be drawn after obtaining informed consent but all other information will be collected from the medical record.

6. Study Subjects

NICU patients (“proband”) and their parents (to form a “trio”) will be enrolled. We will exclude those who were admitted secondary to maternal factors. Baseline data will be drawn from NICU admissions in 2015 during which traditional diagnostic testing was ordered and performed.

7. Recruitment

Potential NICU subjects and their parents will be identified by study staff and consent will be obtained after introduction by the primary NICU attending.

8. Informed Consent Process

Informed consent will be obtained by trained study staff after prospective subjects are first approached by the primary NICU attending. Neonates will be enrolled by their guardian.

9. Confidentiality of Study Data

Unique identifiers will be used for all data collected for the study. The samples sent for sequencing will be labeled with the unique study code and the individuals in the sequencing laboratory will not have access to the link to the subject identity.

10. Privacy Protections

Confidentiality will be protected by collecting only information needed to assess study outcomes, minimizing to the fullest extent possible the collection of any information that could directly identify subjects, and maintaining all study information in a secure manner.

11. Potential Risks

The risks associated with the proposed project include loss of confidentiality and risks associated with venipuncture. All study personnel will be trained in accordance with HIPAA guidelines and blood draws will be done by trained CUMC staff.

12. Data and Safety Monitoring

As this study is non-interventional and requires nothing more than obtaining data and blood/ buccal/ saliva samples on study subjects, no safety events are expected to occur.

13. Potential Benefits

Study participants are not guaranteed any benefit from this research study. It is possible that some number of subjects may benefit from study participation in that they may be provided with a genetic diagnosis although even in the presence of a genetic diagnosis it is unlikely that clinical outcomes will change.

14. Alternatives

The alternative is not to participate in the study.

15. Research at External Sites

No external sites will enroll study subjects into this research study.

16. Columbia as Lead Institution

n/a

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