Proposed Protocol for MPSE: Real-time identification of critically ill newborns most likely to benefit from rWGS.

Automatic prioritization of NICU patients for rWGS. The NICU is an under-recognized reservoir of rare Mendelian diseases that disproportionately contributes to infant mortality.[1,2] The GEMINI study recently established rWGS as the single best test to achieve a rapid genetic diagnosis in the NICU.[3] rWGS diagnosed 30–56% of selected NICU cases and impacted clinical care and resource utilization, with 2/3 of genomic diagnoses leading to changes in clinical management.[4-11] Financial barriers are also being removed with insurers reimbursing rWGS testing,[12] including Utah Medicaid and Select Health, Utah's largest private insurer (see Letter). rWGS is now the standard of care for NICU babies with complex phenotypes.[3,4,7]

MPSE provides an automated means for continuous surveillance of a NICU to identify patients likely to have undiagnosed Mendelian diseases. To evaluate the feasibility and performance of MPSE, we partnered with Rady Children's Hospital to analyze 1,075 Level IV NICU admits, their clinic notes, and metadata such as age and sex. 294 of these children had been selected by Rady clinicians for rWGS, and 84 were diagnosed with Mendelian diseases. Our validation dataset was composed of 2,965 UU NICU admits, and 35 WGS probands sequenced by the UU NeoSeq program.[13] The MPSE Pipeline uses clinical NLP to distill the contents of clinical notes into machine readable Human Phenotype Ontology (HPO) descriptions for every NICU patient. **Figure 4A** shows an MPSE Phenotype description for an actual proband. The HPO terms deemed most predictive for rWGS by MPSE are shown in red, e.g., *Epileptic Encephalopathy.* HPO terms deemed less indicative for rWGS by MPSE (lower scores) are smaller, and tend toward the indigo end of the spectrum, e.g., *Cough*. This proband was diagnosed by rWGS with a female-specific X-linked Epileptic Encephalopathy (OMIM 300088).[14]

MPSE is effective and transportable. After being trained on the clinical notes from Rady Children's NICU, MPSE proved highly effective for identifying and prioritizing UU NICU probands for rWGS—an orthogonal and independent validation. See **Figure 4B** and Peterson *et al.*[14] for details. **Figure 4C** shows diagnostic rates for sequenced probands as a function of MPSE scores; UU probands with a score in the top 50% had a 62% diagnostic rate. Clearly, MPSE can be used to identify the patients most likely to have a genetic diagnosis.

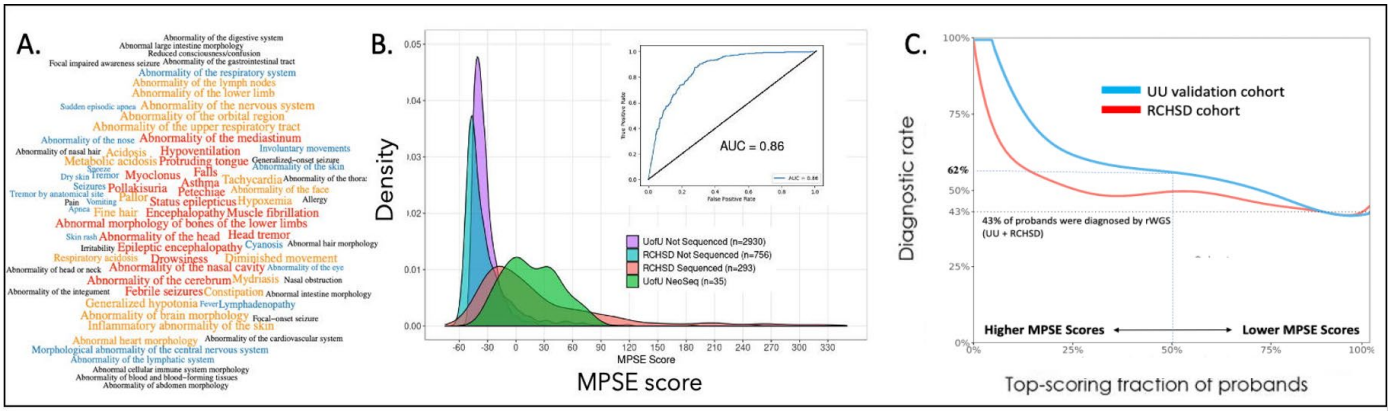

Figure 4. MPSE automatically prioritizes probands for rWGS. A. An automatically generated HPO-based phenotype description scored by MPSE. In this word-cloud, size and color are proportional to each HPO term's contribution to the proband's MPSE score. **B**. Automatic identification of probands with Mendelian phenotypes and rWGS prioritization using NLP-derived HPO descriptions. Distributions of MPSE scores for Rady Children's sequenced (red) and unsequenced (blue) probands. Score distributions for Utah NeoSeq (green) and unsequenced probands (purple). Insert: Receiver Operator Characteristic (ROC) curve. MPSE Scores are -log likelihood ratios. **C**. MPSE diagnostic rates. Sequencing the top 50% of MPSE prioritized probands results in a 62% diagnostic rate.

Impact of Operational Clinical Deployment. Rady Children's has operationally deployed MPSE in 6 of its NICUs as QI, and the UU is preparing to do so currently. Rady's QI deployment has reduced the time to rWGS order by 38% (from 114 to 71 hours), a crucial advancement to institute life-saving therapies in this critically ill population. Collectively, these results demonstrated that MPSE effectively prioritizes probands for rWGS, and that probands with the highest MPSE scores have diagnosable Mendelian conditions.

Pilot deployment of MPSE at selected gLHS Network sites. We anticipate most Network sites will have a

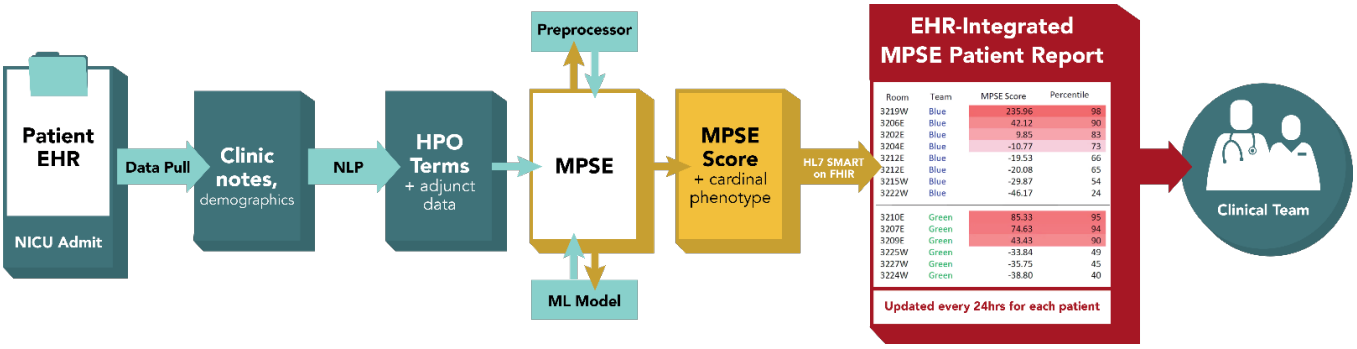

Figure 5. Proposed MPSE workflow. Automated means for continuous surveillance, identification, and prioritization of patients with likely Mendelian diseases for rWGS. The MPSE patient report will be accessible as a SMART on FHIR app by gLHS Network NICUs.

level IV NICU utilizing rWGS when indicated. Here, we propose a pilot deployment of MPSE in the NICUs of participating Network sites to test the feasibility and impact of MPSE implementation across diverse sites. The workflow (**Figure 5**) includes use of an off-the-shelf NLP tool that converts clinical notes into HPO terms and calculates MPSE scores and percentile ranks on a daily basis. If a Network site does not already have an HPO extraction NLP tool in place, an open-source tool such as ClinPhen can be used.[15] The daily MPSE report (which includes patient information, MPSE score and percentile) will be accessed by the NICU team through an EHR-integrated SMART on FHIR app to guide rWGS testing in the context of the patient's clinical condition.

Implementation and Evaluation. The GLUE team will supervise the QI deployment of the MPSE pipeline at participating gLHS Network centers, working collaboratively with local stakeholders (NICU, administrative and IT staff) to overcome local IT and institutional barriers, building on our experience with deployment at Rady and UU. We propose a 2-phase study, each lasting 1.5 years, in which MPSE is used in the NICU to guide rWGS.

Between phases 1 and 2, the lessons learned from phase 1 (e.g., via provider interviews) will be incorporated into an enhanced intervention for phase 2 re-implementation, as will advances in underlying technologies.

The primary outcome will be the number of hours between NICU admission and rWGS testing (time-to-test order). Secondary outcomes will include time to genetic diagnosis, testing rate, diagnostic yield and the number of genetic diagnoses. Our primary analysis will apply linear regression to compare the mean time-to-test order, conditional on a test order within 7 days, between three time periods: Phase 1, Phase 2 and a baseline period of roughly 18 months. The model will include adjustment for patient level covariates. Sensitivity analyses will extend the regression model by including an additional covariate for calendar time to control for linear secular trend. If further nonlinear secular trends are evident, cubic splines in time may be considered, recognizing this would reduce the statistical power of the analysis to detect effects. Similar regression analyses will be applied to compare secondary outcomes between the study periods, using linear regression of quantitative outcomes, logistic regression for binary outcomes including the occurrence of diagnostic testing within 7 days as a dichotomous variable, and negative binomial regression for count outcomes.

Based on our preliminary data, we estimate a standard deviation in time-to-testing of 48 hours. We allow for a 10% inflation in the variance of the estimated treatment effects due to possible imbalances in covariates between the time periods being compared. Assuming 539 NICU admissions and 40% test ordering within 7 days per year, our primary analysis will have 80% power with 2-sided $\alpha=0.017$ (where we have divided 0.05 by 3 to account for three comparisons) to detect a difference of 12.9 hours in mean time-to-test between Phase 1 and baseline, Phase 2 and baseline, or between Phase 2 and Phase 1.

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