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Estimating the sensitivity of genomic newborn screening for treatable inherited metabolic disorders

Authors:

Sarah L. Bick,[1] Aparna Nathan,[2] Hannah Park,[3] Robert C. Green,[4] Monica H. Wojcik,[5,†] Nina B. Gold[6,*,†]

Affiliations:

[1]Division of Genetics and Genomics, Boston Children's Hospital, Boston, MA, 02115; Division of Medical Genetics and Metabolism, Massachusetts General Hospital for Children, Boston, MA, 02114; Harvard Medical School, Boston, MA, 02115.

[2]Department of Biomedical Informatics, Harvard Medical School, Boston, MA, 02115.

[3]Division of Newborn Medicine, Division of Genetics and Genomics, Department of Pediatrics, Boston Children's Hospital, Boston, MA, 02115; Harvard Medical School, Boston, MA, 02115.

[4]Mass General Brigham, Boston, MA, 02114; Broad Institute of MIT and Harvard, Cambridge, MA, 02142; Ariadne Labs, Boston, MA, 02215; Harvard Medical School, Boston, MA, 02115.

[5]Division of Newborn Medicine, Division of Genetics and Genomics, Boston Children's Hospital, Boston, MA, 02115; Harvard Medical School, Boston, MA, 02115; Broad Institute of MIT and Harvard, Cambridge, MA, 02142.

[6]Division of Medical Genetics and Metabolism, Massachusetts General Hospital for Children, Boston, MA, 02115; Harvard Medical School, Boston, MA, 02115.

Correspondence email: *ngold@mgh.harvard.edu, 617-610-3256

Additional footnotes: †Senior authors.

Abstract

Introduction: Over 30 research groups and companies are exploring newborn screening using genomic sequencing (NBSeq), but the sensitivity of this approach is not well understood.

Methods: We identified individuals with treatable inherited metabolic disorders (IMDs) and ascertained the proportion whose DNA analysis revealed explanatory deleterious variants (EDVs). We examined variables associated with EDV detection and estimated the sensitivity of "DNA-first" NBSeq. We further predicted the annual rate of true positive and false negative NBSeq results in the United States for several conditions on the Recommended Uniform Screening Panel (RUSP).

Results: We identified 635 individuals with 80 unique IMDs. In univariate analyses, Black race (OR = 0.37, 95% CI: 0.16-0.89, p = 0.02) and public insurance (OR = 0.60, 95% CI: 0.39-0.91, p = 0.02) were less likely to be associated with finding EDVs. Had all individuals been screened with NBSeq, the sensitivity would have been 80.3%. We estimated that between 0 and 649.9 cases of RUSP IMDs would be missed annually by NBSeq in the United States.

Conclusions: The overall sensitivity of NBSeq for treatable IMDs is estimated at 80.3%. That sensitivity will likely be lower for Black infants and those who are on public insurance.

Keywords: Newborn screening, genomic sequencing, inherited metabolic diseases, detection sensitivity, explanatory deleterious variants

Introduction

Newborn screening (NBS) is a successful public health program that identifies infants at risk for a range of treatable, childhood-onset conditions. The majority of the conditions on the United States Recommended Uniform Screening Panel (RUSP)[1] are inherited metabolic disorders (IMDs), a group of genetic conditions characterized by impaired energy production or pathologic accumulation of toxic metabolites. Collectively, IMDs have an estimated incidence of 1 in 2,500-5,000 live births and lead to significant morbidity and mortality, especially in pediatric populations.[2] NBS labs primarily use tandem mass spectrometry to identify biomarkers of IMDs, but prior studies have suggested that biochemical screening in combination with genomic sequencing may improve the sensitivity and specificity of NBS.[3] In recent years, many NBS labs have added DNA sequencing as a second-tier test to further clarify infants' risk for a range of disorders.[4]

Over 30 research groups and companies around the world are offering genomic sequencing as a first-tier screening test to identify apparently healthy infants at risk for genetic disorders.[5,6] Newborn and childhood screening using genomic sequencing (NBSeq) offers an efficient method to screen simultaneously for hundreds of treatable genetic disorders.[7] Rare disease experts have recognized IMDs as a high-priority group of disorders to include in future NBSeq,[8] and estimates of the sensitivity of NBSeq for treatable conditions will be essential.

Prior studies have addressed the yield of positive results and the positive predictive value of genomic screening in newborns,[9,10] or compared the sensitivity of biochemical and NBSeq for conditions on the RUSP. [3,11] The sensitivity of NBSeq for the detection of a wide range of IMDs, however, remains unknown, and the characteristics of infants who might not be detected by this modality have not yet been characterized. In this study, using treatable IMDs as a class of

diseases for which highly accurate and specific biochemical diagnosis is possible, we explored the estimated sensitivity of NBSeq.

We determined the proportion of individuals at a large academic medical center with clinical diagnoses of IMDs in whom genetic testing did not reveal explanatory deleterious variants (EDVs). We then examined individual and disease-related factors associated with the likelihood of having EDVs. Finally, by combining our results with data from gnomAD v2,[12] we estimated the annual rate of true positive and false negative NBSeq results in the United States for several conditions on the RUSP.

Material and Methods

Study design and case selection

A retrospective chart review was conducted to identify individuals evaluated in the Boston Children's Hospital (BCH) Metabolism Program for known or suspected IMDs over a five-year period from January 1, 2017 to December 31, 2022. The Institutional Review Board of Boston Children's Hospital approved this study (IRB-P00043820).

Inclusion criteria

An initial digital medical records query identified charts for potential study inclusion if individuals: (1) were seen in the study center's outpatient Genetics and Metabolism clinic between the years 2017-2022, (2) were seen by a medical geneticist and/or metabolism physician who evaluates metabolic chief complaints, and (3) were less than 22 years of age (due to limited electronic health records at the study institution prior to 2000).

Charts were further manually reviewed for study inclusion if individuals: (1) had a chief complaint related to the evaluation of a suspected IMD, (2) had undergone both biochemical

and DNA-based testing, (3) were found to have biochemical laboratory test results diagnostic of a specific IMD diagnosis, and (4) had a clinical diagnosis of a treatable IMD that was rendered by a medical geneticist and/or metabolism physician. Chief complaints related to the evaluation of a suspected IMD included: a history of abnormal NBS results, developmental delay, hypotonia, stroke-like episodes, abnormal biochemical test results or radiologic imaging suggestive of an IMD, a known IMD, family history of IMD, dysmorphic physical features, or other.

Treatable IMDs were defined as those with clinically available dietary or pharmaceutical therapy that targets the underlying mechanism of disease.[7] By this definition, treatable IMDs included all core and secondary metabolic conditions on the RUSP[1] as well as 137 metabolic disorders previously curated by Gold et al.[8] We included several additional treatable genetic conditions managed by metabolic physicians at the study institution that are frequently diagnosed or managed by other subspecialties. These conditions included hereditary folate malabsorption (OMIM: 229050), lipoprotein lipase deficiency (OMIM: 238600), metachromatic leukodystrophy (OMIM: 250100), lysosomal acid lipase deficiency (OMIM: 620151), pyridoxine-dependent epilepsy (OMIM: 266100), alkaptonuria (OMIM: 203500), and glycogen storage disease (GSD) types 0 (OMIM: 240600), IV (OMIM: 232500), and V (OMIM: 232600).

Individuals were excluded if biochemical testing or DNA-based testing were incomplete (i.e. ordered or considered but never obtained), harbored only variants known to be associated with enzyme pseudodeficiency,[13] if their diagnosis did not require long-term metabolic care (such as short-chain acyl-CoA dehydrogenase deficiency [OMIM: 201470] or benign hypermethioninemia [OMIM: 250850]), or if their diagnosis was not considered a treatable IMD as described above. Positive biochemical testing was defined as the presence of characteristic lab abnormalities suggestive of a specific IMD.[14] In cases of GSD, which lack a pathognomonic biomarker,

documented hypoglycemia and abnormal liver imaging or an abnormal liver biopsy were considered to be sufficient for a biochemical diagnosis.

The primary outcome measure was whether or not molecular testing revealed EDVs diagnostic of IMD. EDVs were defined as the presence of pathogenic or likely pathogenic variant(s) (PLPVs) in a dosage corresponding to the mode of disease inheritance (i.e. two PLPVs in a gene associated with autosomal recessive inheritance) found on diagnostic genomic testing, such as a gene panel or exome sequencing. This definition is based upon the methodology employed by several newborn genomic sequencing studies in which variants of uncertain significance (VUS) are not reported.[9,15,16,17]

Data collection

Data were abstracted from the electronic medical record (EMR) by two independent reviewers (SB, HP) and were recorded and stored in a secure electronic database hosted by REDCap (Research Electronic Data Capture) electronic data capture tools at BCH.[18,19]

The data collected included demographic information (sex, race, ethnicity, home zip code, insurance status), and clinical information (reason for referral to a metabolism specialist, date of first clinic visit, age at first clinic visit, biochemical testing, and results), and DNA-based sequencing results (gene name, variant, variant classification, IMD diagnosis, IMD classification, year of diagnosis, and clinical status). Race and ethnicity were self-reported by individuals or their families.

The reason for referral was categorized into one of 12 groups: abnormal newborn screen, developmental delay/cognitive impairment, hypotonia, stroke-like episodes/intermittent ataxia/AMS, seizures, failure to thrive/cyclic vomiting, hypoglycemia, abnormal laboratory

results and/or imaging, known IMD diagnosis, family history of IMD, dysmorphic features, and other. IMD classification was based upon the International Classification of Inherited Metabolic Disorders (ICIMD)[20] and included disorders of amino acid metabolism, disorders of carbohydrate metabolism, disorders of fatty acid and ketone body metabolism, disorders of lipid metabolism, disorders of complex molecule degradation, disorders of vitamin and cofactor metabolism, and other types of IMD.

Data analyses

Analyses were conducted using R (version 2023.06.0+421) (R Foundation, Vienna, Austria). Descriptive statistics were calculated to characterize the study cohort, including sex, age at first clinic visit, insurance status, and self- or family-reported race and ethnicity. The proportion of individuals with an established biochemical diagnosis without EDVs was identified, and from this proportion, the predicted sensitivity of NBSeq was estimated.

We designed univariate and multivariable logistic regression models to identify individual and disease characteristics associated with finding EDVs. First, we selected possible predictors that we theorized might influence the likelihood of finding EDVs for a clinically diagnosed IMD, including sex, insurance status, race, ethnicity, RUSP versus non-RUSP conditions, and IMD classification. All variables were categorical, so we modeled them as separate indicator variables for each category. In the univariate models, we used logistic regression (implemented with the glm R function) to associate each indicator variable with the binary outcome of finding EDVs. In the multivariable model, we included all selected variables in the logistic regression model, excluding the most common category of each variable as its reference. We assessed the significance of each variable in each model based on its coefficient Wald p-value. For all quantitative analyses, results were deemed significant at a threshold of $p < 0.05$.

Estimation of sensitivity of genetic testing for IMDs

Using the data from this study, we estimated the hypothetical sensitivity of NBSeq for the identification of the 80 treatable IMDs found in our cohort. For these calculations, individuals with clinical IMD diagnoses and EDVs were considered “true positive” cases. Individuals with clinical diagnoses of IMD but without EDVs were considered “false negative” cases. For each individual, we combined all genetic testing approaches that they had undergone (CMA, familial variant analysis, common variant genotyping, single gene sequencing, gene panel, and exome) as a conglomerate representation of NBSeq.

Estimation of cases missed by NBSeq per year

We then used the estimated sensitivity rates alongside data from gnomAD v2 to calculate the number of cases of IMDs on the RUSP that could potentially be missed by NBSeq annually in the United States.

We first defined the number of infants expected to receive positive NBSeq results for each disorder. The Genetic Prevalence Estimator (GeniE) uses variant databases and population data from ClinVar and gnomAD to estimate the prevalence of deleterious genotypes associated with autosomal recessive diseases (genie.broadinstitute.org).[21] For each disorder on the RUSP, we used the expected prevalence of a deleterious genotype from GeniE as an estimate of the rate of true positive plus false negative NBSeq results.

We defined the rate of false positive NBSeq results as the prevalence of individuals in gnomAD v2 with two deleterious variants known to be in *trans* or unphased. We first tabulated the number of individuals with predicted loss of function variants or likely deleterious missense

variants (REVEL score ≥ 0.932) occurring in *trans* or unphased in the variant co-occurrence tables from gnomAD v2. The false positive rate was calculated by dividing the number of individuals derived from the variant co-occurrence tables by the total number of samples included in gnomAD v2 co-occurrence tables (n=125,748).[22]

To calculate the proportion of infants expected to have true positive NBSeq results, we subtracted the percentage of individuals in gnomAD v2 with two deleterious variants predicted to be in *trans* or unphased from the total prevalence of deleterious variants predicted by GeniE. Multiplying this true positive rate by the number of births in the United States in 2023[23] yielded the predicted annual number of true positive cases detected by genetic testing.

We then used the calculated sensitivity of NBSeq for each RUSP condition from our study and the calculated true positive rate to estimate the total number of affected individuals per year. Subtracting the number of true positive cases detected by genetic testing from this total provided the projected estimate of cases missed by NBSeq annually.

X-linked conditions were omitted from our calculations as GeniE cannot estimate their prevalence. Gene-disease pairs with calculated NBSeq sensitivity of zero or where the predicted false positive rate exceeded the genetic prevalence estimate from GeniE were also excluded.

Results

Descriptive statistics

Among 8,121 charts identified for review by digital medical record query, 635 met criteria for inclusion in these analyses (Figure 1). These individuals had a total of 80 unique treatable IMDs (Supplemental Table 1).

Overall, 55.6% (n=353) of participants were male and 44.4% (n=282) were female, with a median age at presentation of 3.5 months (IQR 13 days - 53.2 months) (Table 1). Most individuals self-identified as White (n = 365, 57.5%) and non-Hispanic (n=439, 69.1%), although 19.2% did not report race and 20.2% (n=128) did not report their ethnicity.

Among all 635 individuals whose DNA had been sent for analysis, 510 (80.3%) were discovered to have EDVs. Disorders of amino acid metabolism made up the largest subset of cases analyzed (n=239, 37.6%), followed by disorders of fatty acid and ketone body metabolism (n=126, 19.8%) (Figure 2). Among all individuals with positive biochemical testing and clinical diagnoses of IMDs, 69.9% (n=444) had IMDs that are included as core or secondary conditions on the RUSP. Of these, 82.2% (n=365) had EDVs and 17.8% (n=79) lacked EDVs.

Of the 510 individuals with EDVs, 396 (77.6%) were diagnosed by single gene testing, 59 (11.6%) were diagnosed by gene panel, 27 (5.3%) were diagnosed by genotyping for common variants, 20 (3.9%) were diagnosed by exome sequencing, 4 (0.8%) were diagnosed by familial variant analysis, and 1 (0.2%) was diagnosed by CMA (Supplemental Table 2). In 3 cases (0.6%), genetic testing was obtained at an outside hospital, and the specific testing modality was unknown.

Of the 125 individuals without EDVs, 78 (62.4%) received single gene testing only, 22 (17.6%) received gene panel testing only, and 5 (4.0%) received exome sequencing only. Multiple genetic tests, including CMA, single gene sequencing, common variant genotyping, gene panel, and exome, were obtained in 20 (16.0%) cases without EDVs. Genome sequencing was not pursued for any individuals in our cohort.

Predictors of EDVs

Individuals of Black/African American race had significantly lower odds of having EDVs compared to White individuals (OR = 0.37, 95% CI: 0.16-0.89). This association remained significant in the multivariable analysis adjusting for all other variables (aOR = 0.32, 95% CI = 0.13-0.87). No other racial groups were associated with significant differences in the odds of having EDVs (Table 2).

Individuals with public insurance as well as those with international/self-pay modes of coverage had lower odds of having EDVs compared to those with private insurance. In the univariate model, the OR for public insurance was 0.60 (95% CI: 0.39-0.91), and the aOR was 0.41 (95% CI: 0.23-0.72). International/self-pay coverage trended towards lower odds of having EDVs in the univariate analysis (OR = 0.41, 95% CI: 0.18-1.05, p = 0.05), but this was not statistically significant in the multivariable analysis (aOR = 0.34, 95% CI: 0.06-2.64, p = 0.23).

Among categories of IMDs, disorders of fatty acid and ketone body metabolism (FAOD) were associated with significantly lower odds of having EDVs compared to the disorders of amino acid metabolism reference group in both the univariate (OR = 0.58, 95% CI: 0.35-0.99) and multivariable analyses (aOR = 0.38, 95% CI: 0.19-0.74). Other IMD classifications did not demonstrate significant associations.

Conditions not included on the RUSP were associated with lower odds of having EDVs compared to those on the RUSP. This association was not significant in the univariate analysis (OR = 0.68, 95% CI: 0.45-1.03, *p* = 0.07) but was significant after adjusting for confounding by the other covariates (aOR = 0.47, 95% CI: 0.23-0.93). None of the other independent variables achieved statistical significance with regard to having EDVs.

Sensitivity of "DNA-first" screening for IMDs

We calculated the sensitivity of NBSeq for IMDs on the RUSP to be 82.2% (365 true positive cases/(365 true positive cases + 79 false negative cases)). Comparatively, 30.1% (n=191) of individuals in this study had been clinically and biochemically diagnosed with an IMD that is not listed as a core or secondary condition on the RUSP. We calculated the sensitivity of NBSeq for 46 treatable IMDs that are not on the RUSP to be 75.9% (145 true positive cases/(145 true positive cases + 46 false negative cases)).

Cumulatively, for all treatable IMDs included in this study, the sensitivity of NBSeq was estimated at 80.3% (510 true positive cases/(510 true positive cases + 125 false negative cases)). Based on the results of this study, if NBSeq were used to expand the number of treatable IMDs assessed by NBS, approximately 19.7% of individuals with biochemical and clinical diagnoses of treatable IMDs might receive false negative results.

Predicted missed cases per year by NBSeq

We estimated the annual number of cases in the US of 18 treatable IMDs on the RUSP that would be missed by NBSeq (Table 3). NBSeq is projected to result in the fewest missed cases of mucopolysaccharidosis I (0.6) (OMIM: 607014), citrullinemia type I (1.7) (OMIM: 215700), and maple syrup urine disease (2.2) (OMIM: 248600, 620698, 620699). NBSeq is expected to miss the highest number of cases of profound/partial biotinidase deficiency (649.9) (OMIM:

253260), Pompe disease (54.4) (OMIM: 232300), and medium-chain acyl-CoA dehydrogenase deficiency (53.0) (OMIM: 201450). No missed cases were expected for malonyl-CoA decarboxylase deficiency (OMIM: 248360), tyrosinemia type I (OMIM: 276700), carnitine palmitoyltransferase II deficiency (OMIM: 600649, 608836, 255110), 3-ketothiolase deficiency (OMIM: 203750), dihydropteridine reductase deficiency (OMIM: 261630), and cobalamin C deficiency (OMIM: 277400).

Discussion

Incorporating NBSeq into population-wide NBS programs has the potential to be lifesaving for infants at risk of treatable genetic disorders.[24] However, the clinical utility of NBSeq as a screening tool will depend, in part, upon its sensitivity—the rate at which it detects infants who will eventually show symptoms of disease. IMDs, which are largely childhood-onset and can be confirmed by biochemical testing, serve as a model to estimate NBSeq's sensitivity. In this study, we reviewed the EMRs of 635 individuals with 80 unique IMDs. We estimated NBSeq to have an aggregate sensitivity of 80.3%, with lower sensitivity expected among infants who were Black and those who were on public insurance. We estimated that annually between 0 and 650 infants in the United States with one of a subset of IMDs would be missed by NBSeq. As international NBSeq efforts expand, our findings highlight their current capabilities for detecting treatable IMDs and suggest ways to improve upon sensitivity in the future.

Traditional NBS methods, including tandem mass spectrometry and enzymatic assays, have a near-perfect sensitivity for many treatable IMDs.[25] Prior analyses have found NBSeq less sensitive than traditional NBS for IMDs on the RUSP. Among 1,728 dried blood spots from infants with IMDs in California, traditional NBS had a sensitivity of 99.0%, whereas exome sequencing had a sensitivity of only 88%.[3] Similarly, the NEXUS study found EDVs in only 15/17 (88%) of infants with phenylketonuria (OMIM: 261600) and medium-chain acyl-CoA

dehydrogenase deficiency, which are rarely missed by traditional NBS.[26] In this study, genetic testing identified EDVs in 82.2% (365/444) of individuals with an IMD included on the RUSP, reinforcing the need to use biochemical testing as the gold standard in screening. However, this study includes IMDs beyond those on the RUSP, allowing for broader assertions about the sensitivity of NBSeq for disorders that cannot be ascertained by traditional biochemical methods.

NBSeq can expand upon the number of IMDs included in NBS. Biochemical testing alone is inaccurate for some treatable IMDs, such as ornithine transcarbamylase deficiency (OMIM: 311250),[27] or infeasible for others, like mitochondrial disorders, which have no single pathognomonic biomarker, or acute intermittent porphyria (OMIM: 176000), which requires specialized testing that may only be positive in times of metabolic crisis. We found that the estimated sensitivity of NBSeq for 46 non-RUSP IMDs was 75.9%. This is slightly lower than for RUSP conditions, likely due to decades of population-level ascertainment of those conditions that has led to more accurate genotype-phenotype correlations. Our estimation of sensitivity for RUSP and non-RUSP conditions was also further influenced by the study cohort size and disease representation within this single hospital system, which can be improved upon in future studies. Importantly, none of the individuals with non-RUSP conditions in this study were identified by current NBS methods, thereby delaying their care until after the emergence and recognition of symptoms. Taken together, the sensitivity of NBSeq is therefore expected to have a greater aggregate sensitivity across all IMDs.

Determining which variants are reported as “deleterious” from NBSeq will affect the sensitivity of this approach. We defined EDVs as PLPVs in a dosage corresponding to the mode of disease inheritance, which is consistent with the methodology of several NBSeq research programs.[9,15,16] FAOD, the second-largest category of IMD in our cohort, had a high proportion

of VUS in clinically diagnosed individuals. If a broader criterion was used, in which one PLPV and one VUS for an autosomal recessive condition constituted EDVs, 58 additional cases in our study would have been classified as true positives. This definition of EDVs would have increased the cumulative sensitivity of NBSeq for IMDs from 80.3% to 89.4%. However, this more lenient approach to variant reporting must be balanced against the risk of increasing the rate of false positive results, which may overwhelm both parents and the pediatric workforce.

We also combined our findings with gnomAD v2 data to estimate the expected annual rate of RUSP IMD cases that might be missed by NBSeq. These theoretical data can be improved upon by large, longitudinal studies, but provide an early estimate of true positive and false negative results from NBSeq. Disorders such as biotinidase deficiency and phenylketonuria, which are relatively more common than other IMDs, were estimated to be more likely to be missed by NBSeq. In part, these findings may reflect the incomplete penetrance and variable expressivity of these disorders, or possibly the presence of causative intronic variants. However, relatively few children with each condition would be missed by NBSeq, and these false negative rates could be easily improved by concurrent or serial biochemical screening when available.

NBSeq may improve upon the positive predictive value (PPV) of traditional NBS methods. Although biochemical screening has greater than 99% sensitivity, only 0.5-2% of infants with positive results are found to have diagnostic evidence of disease because each disorder is individually so rare.[25] To establish the PPV of NBSeq, infants with PLPVs need longitudinal evaluations to detect signs and symptoms of disease. Since genes associated with monogenic disease are less tolerant of loss-of-function variants,[12,28] an assertion supported by our exploration of variant co-occurrence across IMD genes, we expect few false positive results from NBSeq.[29] In a prior analysis, we found that less than 1% of apparently healthy participants in gnomAD (59/141,456) had PLPVs in 127 genes associated with treatable childhood-onset

disorders.[30] This low false positive rate, which is inversely related to the PPV, suggests that genomic sequencing may offer more prognostic information than current biochemical screening methods alone.

NBSeq can mitigate healthcare disparities related to referral patterns and access to care, but limitations remain.[31] Individuals of Black/African American race had lower odds of having EDVs compared with those who were White, a disparity seen in prior studies.[32,33] Non-White participants in large genomic databases have fewer pathogenic variants and more VUS than White participants.[34] This discrepancy may be mitigated by including parental samples in genome sequencing analysis, as shown in the 100,000 Genomes Project from Genomics England.[35] This approach may not be feasible for NBS, as samples from both parents may not always be available. As more data from more diverse individuals becomes available in large genomic databases, analysis of proband-only sequencing will improve.

The sensitivity of genetic testing is likely to grow with time. Over half (62.4%) of individuals without EDVs in this cohort had single-gene testing, which may miss causative variants in genes with similar biochemical signatures. Variants in non-coding regions may also go undetected by exome or panel sequencing.[36] Genome sequencing and RNAseq, which were not used in our cohort, have an 8% and 15% greater diagnostic yield compared with exome alone.[37–39] However, a recent analysis showed that many children with signs of IMD who had negative exome testing still lacked a diagnosis after exome reanalysis and genome sequencing.[39] With time, the analysis and interpretation of coding and non-coding variants will continue to improve.

While traditional biochemical screening outperforms NBSeq for many disorders of primary metabolism, it cannot efficiently detect hundreds of other childhood-onset treatable disorders. The interpretation of NBSeq results is limited by the lack of longitudinal population-wide

phenotype data and longstanding disparities in genomics research, but its yield appears high and is consistently improving. With ongoing gene discovery, variant curation efforts, and increasing availability of genome sequencing, NBSeq's sensitivity and PPV will continue to grow, eventually enabling the detection of many more children at risk for treatable IMDs.

Data Availability

Data in a de-identified format will be made available by request to the corresponding author.

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Author Contributions

The authors confirm contribution to the paper as follows: Conceptualization: S.L.B., M.H.W., and N.B.G.; Data curation: S.L.B., H.P., and M.H.W.; Formal analysis: S.L.B., A.N., M.H.W., and N.B.G.; Funding acquisition: N.B.G.; Investigation: S.L.B., M.H.W., and N.B.G.; Methodology: S.L.B., A.N., M.H.W., and N.B.G.; Resources: R.C.G.; Software: A.N.; Supervision: R.C.G., M.H.W., and N.B.G.; Visualization: S.L.B. and N.B.G.; Writing - original draft: S.L.B.; Writing - review & editing: R.C.G., M.H.W., and N.B.G.

Ethics Declaration

The Institutional Review Board of Boston Children's Hospital approved this study (IRB-P00043820). The research could not practicably be conducted without the waiver of informed consent and authorization because it was not feasible to consent all individuals to enroll in a retrospective study. Moreover, individuals included in this study may have been deceased, lost to follow-up, or may not have accurate contact information.

Declaration of Interests

R.C.G. receives compensation for advising the following companies: Allelica, Atria, Fabric, Genomic Life, and Juniper Genomics; and is co-founder of Genome Medical and Nurture Genomics. M.H.W. has consulted for Sanofi and Illumina and received speaking honoraria from Illumina and GeneDx. N.B.G. has consulted for RCG Consulting and received speaking honoraria from Ambry Genetics.

Supplemental File Listing

Supplemental Table 1. Unique IMD diagnoses included in this study.

Supplemental Table 2. Genetic testing and results.

References

1. Recommended uniform screening panel. Accessed January 5, 2024. https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp

2. Sanderson S, Green A, Preece MA, Burton H. The incidence of inherited metabolic disorders in the West Midlands, UK. *Arch Dis Child*. 2006;91(11):896-899.

3. Adhikari AN, Gallagher RC, Wang Y, et al. The role of exome sequencing in newborn screening for inborn errors of metabolism. *Nat Med*. 2020;26(9):1392-1397.

4. Luo X, Wang R, Fan Y, Gu X, Yu Y. Next-generation sequencing as a second-tier diagnostic test for newborn screening. *J Pediatr Endocrinol Metab*. 2018;31(8):927-931.

5. Downie L, Bouffler SE, Amor DJ, et al. Gene selection for genomic newborn screening: moving towards consensus? *Genet Med*. Published online January 23, 2024:101077.

6. Minten T, Adelson S, Amendola L, et al. P213: Exploring heterogeneity among gene lists proposed for newborn sequencing. *Genetics in Medicine Open*. 2024;2:101110.

7. Bick D, Bick SL, Dimmock DP, Fowler TA, Caulfield MJ, Scott RH. An online compendium of treatable genetic disorders. *Am J Med Genet C Semin Med Genet*. 2021;187(1):48-54.

8. Gold NB, Adelson SM, Shah N, et al. Perspectives of Rare Disease Experts on Newborn Genome Sequencing. *JAMA Netw Open*. 2023;6(5):e2312231.

9. Holm IA, Agrawal PB, Ceyhan-Birsoy O, et al. The BabySeq project: implementing genomic sequencing in newborns. *BMC Pediatr*. 2018;18(1):225.

10. Green RC, Shah N, Genetti CA, et al. Actionability of unanticipated monogenic disease risks in newborn genomic screening: Findings from the BabySeq Project. *Am J Hum Genet*. 2023;110(7):1034-1045.

11. Wojcik MH, Zhang T, Ceyhan-Birsoy O, et al. Discordant results between conventional newborn screening and genomic sequencing in the BabySeq Project. *Genet Med*. 2021;23(7):1372-1375.

12. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581(7809):434-443.

13. Chen-Deutsch X, Donti T, Lin Z, Collins C, Hegde M. P441: Genomic and biochemical profile of pseudodeficiency in lysosomal storage disorders. *Genetics in Medicine Open*. 2023;1(1):100488.

14. Lee JJY, Wasserman WW, Hoffmann GF, van Karnebeek CDM, Blau N. Knowledge base and mini-expert platform for the diagnosis of inborn errors of metabolism. *Genet Med*. 2018;20(1):151-158.

15. Rahman S, Bick D, Scott RH. Whole genome sequencing to screen 100 000 newborns for treatable genetic disorders. *J Inherit Metab Dis*. 2024;47(1):7-8.

16. Kingsmore SF, Smith LD, Kunard CM, et al. A genome sequencing system for universal

newborn screening, diagnosis, and precision medicine for severe genetic diseases. *Am J Hum Genet*. 2022;109(9):1605-1619.

17. Ceyhan-Birsoy O, Murry JB, Machini K, et al. Interpretation of Genomic Sequencing Results in Healthy and Ill Newborns: Results from the BabySeq Project. *Am J Hum Genet*. 2019;104(1):76-93.

18. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381.

19. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform*. 2019;95:103208.

20. Ferreira CR, Rahman S, Keller M, Zschocke J, ICIMD Advisory Group. An international classification of inherited metabolic disorders (ICIMD). *J Inherit Metab Dis*. 2021;44(1):164-177.

21. Genetic Prevalence Estimator. Accessed June 20, 2024. http://genie.broadinstitute.org

22. Variant co-occurrence (phasing) information in gnomAD. Accessed June 21, 2024. https://gnomad.broadinstitute.org/news/2021-07-variant-co-occurrence-phasing-information-in-gnomad/

23. Hamilton BE, Martin JA, Osterman MJK. Births: Provisional Data for 2023. Published online April 25, 2024. doi:10.15620/cdc:151797

24. Stark Z, Scott RH. Genomic newborn screening for rare diseases. *Nat Rev Genet*. 2023;24(11):755-766.

25. Kwon C, Farrell PM. The magnitude and challenge of false-positive newborn screening test results. *Arch Pediatr Adolesc Med*. 2000;154(7):714-718.

26. Roman TS, Crowley SB, Roche MI, et al. Genomic Sequencing for Newborn Screening: Results of the NC NEXUS Project. *Am J Hum Genet*. 2020;107(4):596-611.

27. Staretz-Chacham O, Daas S, Ulanovsky I, et al. The role of orotic acid measurement in routine newborn screening for urea cycle disorders. *J Inherit Metab Dis*. 2021;44(3):606-617.

28. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;536(7616):285-291.

29. Stenton SL, Campagna M, Philippakis A, O'Donnell-Luria A, Gelb MH. First-tier next-generation sequencing for newborn screening: An important role for biochemical second-tier testing. *Genetics in Medicine Open*. 2023;1(1):100821.

30. Gold NB, Harrison SM, Rowe JH, et al. Low frequency of treatable pediatric disease alleles in gnomAD: An opportunity for future genomic screening of newborns. *HGG Adv*. 2022;3(1):100059.

31. Fraiman YS, Wojcik MH. The influence of social determinants of health on the genetic diagnostic odyssey: who remains undiagnosed, why, and to what effect? *Pediatr Res*.

2021;89(2):295-300.

32. Landry LG, Ali N, Williams DR, Rehm HL, Bonham VL. Lack Of Diversity In Genomic Databases Is A Barrier To Translating Precision Medicine Research Into Practice. *Health Aff* . 2018;37(5):780-785.

33. Sirugo G, Williams SM, Tishkoff SA. The Missing Diversity in Human Genetic Studies. *Cell*. 2019;177(1):26-31.

34. Sun KY, Bai X, Chen S, et al. A deep catalogue of protein-coding variation in 983,578 individuals. *Nature*. Published online May 20, 2024. doi:10.1038/s41586-024-07556-0

35. England G. 100,000 Genomes Project: Exploring the diagnosis of rare conditions in the context of genetic ancestry. Genomics England. Published December 9, 2022. Accessed May 21, 2024. https://www.genomicsengland.co.uk/blog/rare-conditions-diagnosis-genetic-ancestry

36. Soriano-Sexto A, Gallego D, Leal F, et al. Identification of Clinical Variants beyond the Exome in Inborn Errors of Metabolism. *Int J Mol Sci*. 2022;23(21). doi:10.3390/ijms232112850

37. Ewans LJ, Minoche AE, Schofield D, et al. Whole exome and genome sequencing in mendelian disorders: a diagnostic and health economic analysis. *Eur J Hum Genet*. 2022;30(10):1121-1131.

38. Peymani F, Farzeen A, Prokisch H. RNA sequencing role and application in clinical diagnostic. *Pediatr Investig*. 2022;6(1):29-35.

39. Wojcik MH, Lemire G, Berger E, et al. Genome Sequencing for Diagnosing Rare Diseases. *N Engl J Med*. 2024;390(21):1985-1997.

Figure Legends

Figure 1. Methods flow diagram. Abbreviations: IMD, inherited metabolic disease.

Figure 2. Results of genetic testing. Abbreviations: EDV, explanatory deleterious variant; VUS, variants of uncertain significance.

Table 1. Demographic and disease characteristics of patients included in study.

Characteristic	N (%)
Total Cases	635
Sex	
Male	353 (55.6)
Age at Presentation, [IQR]	3.2 months, [13 days – 53.2 months]
Race	
Additional races	92 (14.5)
Asian	33 (5.2)
Black or African American	23 (3.6)
White	365 (57.5)
Unknown	122 (19.2)
Ethnicity	
Hispanic	68 (10.7)
Non-Hispanic	439 (69.1)
Unknown	128 (20.2)
Insurance Status	
Public	243 (38.3)
Private	342 (53.9)
International/self-pay	26 (4.1)
Unknown	24 (3.8)
IMD Classification	
Disorders of amino acid metabolism	239 (37.6)
Disorders of carbohydrate metabolism	101 (15.9)
Disorders of fatty acid and ketone body metabolism	126 (19.8)
Disorders of lipid metabolism	22 (3.5)
Disorders of complex molecule degradation	93 (14.6)
Disorders of vitamin and cofactor metabolism	45 (7.1)
Other	9 (1.4)

Abbreviations: IQR, interquartile range; IMD, inherited metabolic disorder. The Additional Races category combines American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, and Other races.

Table 2. Associations of patient characteristics with EDVs.

	Univariate Analysis		Multivariable Analysis	
Characteristic	**OR (95% CI)**	***P* value**	**aOR (95% CI)**	***P* value**
Sex				
Female	1 [Reference]	NA	1 [Reference]	NA
Male	0.83 (0.55-1.23)	0.37	0.92 (0.53-1.56)	0.74
Race				
White	1 [Reference]	NA	1 [Reference]	NA
Additional races	1.43 (0.80-2.72)	0.25	1.53 (0.67-3.74)	0.33
Asian	1.82 (0.70-6.24)	0.27	1.34 (0.48-4.79)	0.62
Black or African American	0.37 (0.16-0.89)	0.02	0.32 (0.13-0.87)	0.02
Ethnicity				
Non-Hispanic	1 [Reference]		1 [Reference]	NA
Hispanic	1.37 (0.70 - 2.95)	0.38	1.42 (0.59-3.68)	0.45
Insurance				
Private	1 [Reference]	NA	1 [Reference]	NA
Public	0.60 (0.39-0.91)	0.02	0.41 (0.23-0.72)	0.002
International/self-pay	0.41 (0.18-1.05)	0.05	0.34 (0.06-2.64)	0.23
IMD Classification				
AA	1 [Reference]		1 [Reference]	NA
Carb	1.02 (0.56 - 1.94)	0.94	1.95 (0.77-5.53)	0.18
FAOD	0.58 (0.35 - 0.99)	0.04	0.38 (0.19-0.74)	0.005
Lipid	0.55 (0.21 - 1.61)	0.24	0.69 (0.22-2.39)	0.54
Complex molecule	0.76 (0.35 - 0.99)	0.36	1.69 (0.72-4.19)	0.24
Vitamin/cofactor	1.35 (0.57 - 3.72)	0.53	1.28 (0.46-4.22)	0.66
Other	0.72 (0.17 - 4.98)	0.69	1.82 (0.24-37.54)	0.61
RUSP				
RUSP	1 [Reference]		1 [Reference]	NA
non-RUSP	0.68 (0.45-1.03)	0.07	0.47 (0.23-0.93)	0.03

Abbreviations: EDVs, explanatory deleterious variants; OR, odds ratio; CI, confidence interval; aOR, adjusted odds ratio; NA, not applicable; IMD, inherited metabolic disorders; AA, disorders of amino acid metabolism; Carb; disorders of carbohydrate metabolism; FAOD, disorders of fatty acid and ketone body metabolism; Lipid, disorders of lipid metabolism; Complex molecule, disorders of complex molecule degradation; Vitamin/cofactor, disorders of vitamin and cofactor metabolism; RUSP, Recommended Uniform Screening Panel. The Additional Races category combines American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, and Other races.

Table 3. Predicted number of missed cases per year by genotype-first NBS for RUSP conditions.

Diagnoses	Associated Gene(s)	Sn	GeniE Genetic Prevalence Estimate	gnomAD Variant Co-occurrences	Estimated Total Annual Cases	Missed Cases per Year
Disorders of amino acid metabolism						
ASL deficiency	*ASL*	0.63	1.24451E-05	1	16.1	9.7
Citrullinemia type I	*ASS1*	0.83	2.31862E-06	0	8.3	1.7
3-MCC deficiency	*MCCC1*, *MCCC2*	0.17	2.00181E-06	0	7.2	35.9
Isovaleric acidemia	*IVD*	0.50	1.9798E-06	0	7.1	7.1
Malonyl-CoA decarboxylase deficiency	*MLYCD*	1.00	1.51713E-07	0	0.5	0
MSUD	*DBT*, *BCKDHA*, *BCKDHB*	0.78	2.10817E-06	0	7.6	2.2
PKU/hyperPhe	*PAH*	0.94	0.000128502	1	432.9	29.7
Tyrosinemia type I	*FAH*	1.00	2.11077E-06	0	7.6	0
Disorders of carbohydrate metabolism						
Galactosemia	*GALT*	0.84	1.31933E-05	1	18.8	3.6
Disorders of fatty acid and ketone body metabolism						
CPT II deficiency	*CPT2*	1.00	7.53449E-06	0	27.1	0
MCAD	*ACADM*	0.81	7.23694E-05	1	231.3	53.0
VLCAD	*ACADVL*	0.67	1.11884E-05	0	40.2	20.1
3-ketothiolase deficiency	*ACAT1*	1.00	4.3978E-07	0	1.6	0
Disorders of complex molecule degradation						
MPS I (Hurler syndrome)	*IDUA*	0.70	8.36624E-06	1	1.5	0.6
Pompe disease	*GAA*	0.81	7.37028E-05	1	236.1	54.5
Disorders of vitamin and cofactor metabolism						
DHPR deficiency	*QDPR*	1.00	2.97348E-08	0	0.1	0
Biotinidase deficiency/partial biotinidase deficiency	*BTD*	0.90	0.001628664	0	5849.1	649.9
Cobalamin C deficiency	*MMACHC*	1.00	4.11948E-06	0	14.8	0

Abbreviations: ASL, argininosuccinate lyase; 3-MCC, 3-methylcrotonyl-CoA carboxylase; IVA, isovaleric acidemia; MSUD, maple syrup urine disease; PKU, phenylketonuria; hyperPhe, hyperphenylalaninemia; CPT, carnitine palmitoyltransferase; MCAD, medium-chain acyl-CoA dehydrogenase deficiency; VLCAD, very long-chain acyl-CoA dehydrogenase deficiency; MPS, mucopolysaccharidosis; DHPR, dihydropteridine reductase.