Data-driven consideration of genetic disorders for global genomic newborn screening programs

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Data-driven consideration of genetic disorders for global genomic newborn screening programs

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Structured Abstract

Purpose: Over 30 international studies are exploring newborn sequencing (NBSeq) to expand the range of genetic disorders included in newborn screening. Substantial variability in gene selection across programs exists, highlighting the need for a systematic approach to prioritize genes.

Methods: We assembled a dataset comprising 25 characteristics about each of the 4,390 genes included in 27 NBSeq programs. We used regression analysis to identify several predictors of inclusion, and developed a machine learning model to rank genes for public health consideration.

Results: Among 27 NBSeq programs, the number of genes analyzed ranged from 134 to 4,299, with only 74 (1.7%) genes included by over 80% of programs. The most significant associations with gene inclusion across programs were presence on the US Recommended Uniform Screening Panel (inclusion increase of 74.7%, CI: 71.0%-78.4%), robust evidence on the natural history (29.5%, CI: 24.6%-34.4%) and treatment efficacy (17.0%, CI: 12.3%-21.7%) of the associated genetic disease. A boosted trees machine learning model using 13 predictors achieved high accuracy in predicting gene inclusion across programs (AUC = 0.915, R^2 = 84%).

Conclusion: The machine learning model developed here provides a ranked list of genes that can adapt to emerging evidence and regional needs, enabling more consistent and informed gene selection in NBSeq initiatives.

Keywords: newborn screening; genomic sequencing; gene selection; gene-disorder associations; machine learning

Introduction

A decade ago, the BabySeq Project piloted newborn and childhood sequencing (NBSeq), a process designed to detect risk for a wide range of genetic disorders in apparently healthy infants.[1–9] Per the data repository Rx-Genes, over 700 genetic disorders now have targeted treatments or consensus guidelines for long-term management, which has further fueled enthusiasm for NBSeq.[10,11] Stakeholders, including diverse groups of parents,[12–14] rare disease specialists,[11] primary care physicians,[15] genetic counselors,[11,16,17] and the public[18,19] now support the implementation of genomic newborn screening for at least some disorders. At least 30 international research programs and companies are actively exploring this screening approach,[20–22] most of which are exchanging best practices through the International Consortium on Newborn Sequencing (ICoNS).[23]

Historically, the criteria established by Wilson and Jungner[24] have provided a framework for selecting the disorders to include in public newborn screening programs. These criteria prioritize the inclusion of childhood-onset disorders that are treatable if diagnosed in their earliest stages and require immediate intervention to prevent irreversible damage. Given the hundreds of treatable disorders that could be candidates for NBSeq and the complexity of genomic data, however, selection of the appropriate genes and disorders for NBSeq is a recurring challenge.[21,25,26] Prior studies have identified discrepancies across the genes being analyzed by a limited number of commercial NBSeq programs[27] and research studies,[28,29] but little is known about the values and variables that underlie these differences. Understanding which genes have high concordance across programs may guide emerging NBSeq research programs as they select which genes and variants to report to participants. Furthermore, the characteristics of these genes and their associated disorders can be used more empirically to prioritize candidate genes for public health programs.

To understand the variability among newborn sequencing programs, we compared the genes currently selected for analysis by 27 research studies and commercial NBSeq programs. For each gene that was included in any NBSeq program, we assembled a dataset of 25 associated characteristics. We then used a multivariate regression analysis to identify which of these characteristics were associated with inclusion across programs. Finally, we used a boosted trees model to generate a ranked list of genes, offering a data-driven approach to the prioritization of genetic disorders for population-wide NBSeq.

Methods

Study design

This cross-sectional study involved four stages: (1) identification of gene lists across international NBSeq research studies and commercial programs, (2) compilation of a dataset of characteristics for each gene included in any program, (3) statistical comparison of gene lists across programs, and (4) development of a machine learning model to predict gene inclusion across NBSeq programs based on gene characteristics.

Identification of lists of genes from research studies and commercial programs

A total of 35 NBSeq programs were identified through membership in the International Consortium on Newborn Sequencing (ICoNS) and an online search (Supplementary Table 1, Figure 1).[5,30–47] We obtained gene lists from 27 NBseq programs, including 20 research studies: BabyDetect,[30,31] BabyScreen+,[28] BabySeq,[2] BeginNGS,[32,33] Chen et al. 2023,[34] Early Check,[35,48] FirstSteps, the Generation study, gnSTAR,[36,38] GUARDIAN study,[42,49,50] Jian et al. 2022,[37] Lee et al. 2019,[43] Luo et al. 2020,[44] NeoExome,[47] NeoSeq,[40] NESTS,[41] NewbornsInSA, Progetto Genoma Puglia, Screen4Care,[45] and Wang et al. 2023.[39] Seven lists of genes from commercial firms that offer genomic newborn screening were included: FORESITE 360 (Fore Genomics), Fulgent (Newborn Genetic Analysis), Igenomix (Igenomix newborn screening), Mendelics (Teste da Bochechinha), Nurture Genomics, PerkinElmer Revvity (Genetic InsightPanel),[46] and Sema4 (Natalis).[30] Nine studies reported screening outcomes and we also summarized the positive screen rates and positive predictive values for these studies (Table 1, Supplementary Methods).

Compilation of a dataset of gene-disorder characteristics

All obtained gene lists were aggregated, standardizing gene names to HGNC nomenclature and linking each gene to a single disorder using OMIM and ClinGen (Supplementary Methods). A data repository with 25 characteristics for each gene-disorder pair was created, collecting data from five research papers and five existing databases (see Supplementary Methods).[2,10,11,32,51–53] More information on the source and description of each of the characteristics can be found in the Supplementary Methods and Supplementary Table 3. This data repository as well as the gene list data were made available online in a Github Repository.

Statistical comparison of gene lists across programs

Descriptive statistics for each gene list, including the length of the list, proportion of genes in each clinical category, and the number of genes associated with RUSP conditions were calculated (Figure 2A, 2B, Supplementary Figure 2, and Supplementary Table 4). To provide information on the concordance across all lists of genes, an UpSet plot, Venn Diagrams, and Jaccard similarity indices were provided (Figure 2C and 2D, Supplementary Figure 3 and 4). Inclusion of high concordance genes, as well as genes associated with core and secondary RUSP conditions, were plotted (Figure 2E, 2F, 2G).

A linear regression model was used to identify factors associated with inclusion in multiple gene lists. Two types of regressions were performed: regressions in which the outcome variable is the proportion of gene list inclusion *across all NBSeq programs* (Figure 3A, Supplementary Table 5 and 6) and regressions in which the outcome variable is the inclusion of a gene for each *individual* study (Figure 3B and Supplementary Table 7).

Development of a machine learning model

We developed a machine learning prediction model to prioritize genes for population-wide

NBSeq programs. Out of 25 potential gene-disorder characteristics, we selected 13 characteristics as predictors in our model: the RUSP category, clinical area, evidence base, severity of the disorder, the treatment efficacy, penetrance, treatment acceptability, age of onset, existence of an orthogonal test, the recommendation score, inheritance, prevalence, and the ClinGen Disease Validity. The remaining 12 gene and disease characteristics were excluded due to a high amount of missing data. For example, the ClinGen actionability scores were not used due to their availability for only 242 genes. Additionally, when characteristics from different sources described similar concepts, we selected the characteristic that includes data on the most genes.

Three machine learning models were compared: linear regression, random forest and boosted trees. We randomly split the gene list data into a 80% training set (n=91,312, 80% of all 114,140 potential instances of 4,390 genes included on a gene list across 26 NBSeq programs) and a 20% test set to estimate prediction power of the model. The boosted trees model, which had the highest area under the curve (AUC), was then selected to generate predictions for all 4,390 genes, providing a ranked list of genes (Supplementary Table 8).

Results

Overview of NBSeq programs

Of the 35 research and commercial programs identified, 10 were located in North America, 10 in Asia, nine in Europe, four in Australia and New Zealand, one in South America and one in Africa (Figure 1, Supplementary Table 1). The research programs anticipate a combined total sample size of 560,410 infants, with the intended enrollment in each study varying from 48 to 101,000 infants.[43,54] Nine NBSeq research programs have published or presented the screening results from a collective total of 68,628 infants by September 2024 (Table 1, Supplementary Methods).

[Figure 1 about here]

The percentage of positive screening results ranged from 1.85% in BabyDetect (3,847 infants screened for 405 genes) to 9.43% in BabySeq (159 infants screened for 4,299 genes), with an average of 3.82% positive results across 68,628 infants. There was a significant positive correlation between the percentage of positive screening results in a program and the number of genes they screened (pearson correlation coefficient of 0.653, p=0.041). A majority of the collective 1,937 positive screening results across seven studies for which detailed results were available were due to variants in *G6PD* (56.8%).

[Table 1 about here]

Four studies reported the clinical outcomes of infants who had undergone NBSeq, allowing for the calculation of these studies' positive predictive value (PPV), which varied from 12% to 79%[49,50] with an average across studies of 41% (39% when weighted by sample size).

Description of gene lists across NBSeq programs

A total of 23 programs have published or made available criteria for selecting genes and disorders for screening (Supplementary Table 2). The number of genes included in 27 programs ranged from 134 to 4,299 (median=306) (Figure 2A, 2B). A total of 4,390 genes (out of a total of 4,966 genes associated with human disease)[55] were included across at least one of the 27 gene lists (Supplementary Table 4). Of these, 4,033 genes (91.8%) were associated with a phenotype in the OMIM database. Collectively, most genes were linked to inherited metabolic disorders (IMDs) (25.4%), neurologic (15.5%), and immunologic (11.9%) disorders (Figure 2A, 2B).

[Figure 2 about here]

Discordance among gene lists used in NBSeq programs

A pairwise Jaccard Index indicated that, aside from those from commercial laboratories, most pairs of gene lists from NBSeq research programs were highly discrepant (Figure 2C and 2D, Supplementary Figure 2, 3). Of the 4,390 genes included in at least one NBSeq program, the vast majority were included by only a small number of NBSeq programs: 4,089 genes (93%) were included by 10 or fewer programs and 3,793 (87%) genes were included by five or fewer programs (Supplementary Figure 1).

Genes with high concordance across NBSeq programs

Despite this variability across gene lists, we found 74 genes (1.7% of 4,390) that were included by over 80% (22 of 27) of NBSeq programs (Supplementary Figure 1). Of these genes, 58 were associated with diseases on the US Recommended Uniform Screening Panel (RUSP) (Figure 2E, 2F). A total of 34 genes not linked to disorders on the RUSP appeared on 20 or more lists (Figure 2G).

Predictors of gene inclusion across NBSeq programs

Genes associated with core or secondary disorders on the RUSP were significantly more likely to be included in NBSeq programs (regression coefficient 74.7%, 95% confidence interval (CI): 0.710-0.784, p<0.01; regression coefficient 60.0%, 95% CI: 0.557-0.643, p<0.01, Figure 3A, Supplementary Table 5). Additionally, genes that were recommended for inclusion in newborn screening by 80% or more rare disease experts in a recent survey[11] were 43.5% (95% CI: 37.4%-49.6%, p<0.01) more likely to be included than genes that were recommended by fewer experts (Supplementary Figure 5).

[Figure 3 about here]

A strong predictor of inclusion across NBSeq programs was the ASQM evidence base, previously defined as a metric measuring a combination of gene-disease validity, published descriptions of the natural history of disease, and the availability of practice guidelines for disease diagnosis and management.[51] Genes with the highest evidence base were 29.5% more likely (95% CI: 24.6%-34.4%, p<0.01) to be included in NBSeq programs than those with less available evidence.

Other characteristics associated with inclusion across NBSeq programs were high efficacy of disease treatment (17.0%, 95% CI: 12.3%-21.7%, p<0.01), high penetrance (15.5%, 95% CI: 9.8%-21.2%, p<0.01), neonatal- or infantile-onset (15.2%, 95% CI: 10.7%-19.7%, p<0.01), high disease severity (14.5%, 95% CI: 8.2%-20.8%, p<0.01), high acceptability of treatment (with regard to the burdens and risks placed on the individual) (15.0%, 95% CI: 10.5%-19.5%, p<0.01), and the existence of an non-molecular test that could be used to confirm the diagnosis (13.8%, 95% CI: 9.3%-18.3%, p<0.01). There was variability in the importance of different characteristics across programs, but programs adhered positive value to all these characteristics with few exceptions (Figure 3B and Supplementary Table 7).

Measuring evolving knowledge about genes and diseases

We conducted a multivariate regression analysis to predict how changes in specific variables, such as treatability and evidence base, would individually influence the overall regression (Supplementary Table 6). Notably, the introduction of a new, highly acceptable treatment for a disorder with no previous treatment is associated with an increase in the likelihood of inclusion in NBSeq programs by 9.7% (95% CI: 0.1%-19.3%, p<0.05). Similarly, improving knowledge related to the natural history of a gene-disorder pair from “none” to “perfect” would increase the likelihood of inclusion in NBSeq programs by 14.8% (95% CI: 4.8%-24.8%, p<0.01).

Machine learning prediction model

Of three machine learning methods, the boosted trees model demonstrated the highest accuracy in the hold-out test set, with an area under the curve (AUC) of 0.915 and Rsquared of 80% (n=22,828, 20%) (Figure 3C, 3D). The relative importance of all variables in the boosted trees model was highest for characteristics such as the proportion of experts who recommended inclusion of the gene in NBSeq on a recent survey,[11] RUSP classification, and disease prevalence, confirming the results from the regression analysis (Supplementary Figure 8).

We used the boosted trees model to predict the observed inclusion of genes across all NBSeq programs, resulting in a list of all genes that had appeared in any NBSeq program, ranked by their predicted inclusion probabilities. Given that most genes associated with conditions on the RUSP were highly ranked, a list with these genes redacted was considered to be more illustrative of the novel capabilities of the model (Table 2). This analysis identified five genes *(PTPRC, ACADSB, NHEJ1, CYBA, and GRHPR)* that, despite being highly ranked by the model, were only included in a low proportion of NBSeq programs. To address missing data for some genes that were included across multiple NBSeq programs, we also created a second ranked list that combines the rankings generated by our machine learning model with the proportion of NBSeq programs in which each gene was observed with equal weights (Supplementary Table 8). By integrating these two sources of information, this hybrid list leverages the most comprehensive evidence available to prioritize genes for potential implementation in public health programs.

[Table 2 about here]

Discussion

NBSeq is a rapidly advancing area of global research exploring the impacts of early diagnosis for infants at risk for genetic disorders. With positive screening results in 1.85% to 9.43% of infants and a higher average PPV than some traditional newborn screening techniques,[49,56] findings from NBSeq research programs support the premise that this approach could improve early detection rates for a wide range of treatable disorders.

However, selecting the appropriate genes for screening is a critical step toward implementing population-wide NBSeq. This decision will have significant implications for at-risk infants, their families, and pediatric healthcare systems.[20,21] In this study, we compared and collected data on the genes being analyzed by 27 NBSeq programs, then developed a machine learning model to predict the inclusion of a gene across NBSeq programs. By combining this model with observed data from NBSeq programs, we generated a ranked list of genes that offers a data-driven approach to prioritizing genetic disorders for public health programs looking to incorporate NBSeq into their screening strategies.

Similar to the findings of smaller studies,[28,29] our comparison of gene lists from 27 NBSeq programs revealed substantial heterogeneity, which we probed using a series of regression models. We found that the importance of individual gene and disease characteristics varied across studies, potentially due to differences in the international prevalence of disorders, the availability of specialists and treatments in different countries and healthcare systems, or the specific goals of individual NBSeq programs. Unexpectedly, 357 genes with no disease association on OMIM and 52 with limited or refuted gene-disease validity scores from ClinGen were included by some NBSeq programs, demonstrating variation among programs in willingness to include candidate genes or those with new associations to disease.

Despite variations in the gene lists used by NBSeq programs, many share a common focus on certain clinical areas and specific genes. All programs included a substantial proportion of genes associated with disorders that are on the RUSP, reflecting the potential for genomic sequencing to detect cases missed by traditional newborn screening programs.[57–59] Of note, genes associated with some disorders on the RUSP, such as 3-methyl-crotonyl-CoA carboxylase deficiency (*MCCC1*, *MCCC2*), were widely included across lists despite not conforming to the historic Wilson-Jungner criteria, due to low penetrance and often mild symptoms.[60] This suggests that some NBSeq programs have anchored their lists around the RUSP even when the disorders are neither severe nor highly treatable.[61] Therefore, the observed concordance of a gene across NBSeq programs alone may not be sufficient to evaluate the suitability of a gene for population-wide screening.

Many gene and disease characteristics emerged as highly associated with the inclusion of genes across NBSeq programs, including the strength of published data on the natural history of disease, estimated penetrance, and the effectiveness of the associated treatment. Interestingly, despite the inclusion criteria that several NBSeq programs reported, characteristics such as the age of onset and disease severity were weakly associated with inclusion, possibly due to their subjective nature.

The machine learning model developed in this study identifies the disorders that may be most appropriate for genomic newborn screening, based on 13 characteristics and their inclusion across 27 NBSeq programs. This ranked list, along with the preferences of rare disease experts[11] could be used to prioritize genes for screening, which could then be manually curated by a team of expert reviewers. At this time, the model's predictions reflect a consensus drawn from NBSeq studies and databases, but in the future, these could be combined with hard-coded gene selection criteria. The model's flexibility also allows updates based on regional preferences, new data, or emerging therapeutics.

Importantly, this model identified several genes included by only a few NBSeq programs, but which have characteristics that are highly associated with inclusion across programs. For example, although *PTPRC*, a gene associated with severe combined immunodeficiency (SCID), was only included by 12 of 27 NBSeq programs, the model ranked it 113 of 4,390 genes. This is likely because *PTPRC* is associated with a severe immunologic disorder that typically presents in childhood and can be treated with an early hematopoietic stem cell transplant, but is a rare cause of SCID.[62] This finding highlights the model's potential to identify genes that may have been overlooked by researchers during the gene selection process.

Our study has several limitations. First, some of the NBSeq programs may have dynamic gene lists that have changed over time. Second, the treatability of various conditions varies based on country. Next, the Jaccard index may exaggerate discrepancies between gene lists of different lengths. For the regression and machine learning models, we consolidated metrics including the ASQM, BabySeq, and ClinGen databases, most of which rely on expert-curated information, such as age of onset, for which definitions vary, and data were missing among the 25 characteristics that we collected. These gaps may automatically reduce the boosted tree model's predicted inclusion of genes that are not well-characterized, resulting in lower inclusion rates for genes related to disorders that are rare or have limited published evidence. To mitigate the effects of missing data, we designed the model to be easily updated, and provided a ranked gene list that takes into account observed inclusion in addition to our model estimates. The ranked list may suffer from overfitting, given that it includes genes on which the model was trained. Lastly, the model incorporates data from funded research programs and commercial products, which may not align with the goals or constraints of public health newborn screening programs.

In the future, we plan to further develop the dataset that we have created to improve our machine learning model, by incorporating the perspectives of key informants, such as parents[13,14] and pediatricians. Additional informatics data, such as gene length, gnomAD constraint score,[63] the ENCODE blacklist,[64] or the number of associated PubMed publications, will be updated in future versions. We also plan to develop a method to iteratively add genes with newly established gene-disease validity based on recent publications to our dataset. Future research will identify genes that are not currently included in any NBSeq program, but share similar characteristics to those that are highly represented. A knowledge graph, such as PrimeKG,[65] could be used to find genes that share common molecular pathways, treatments, or symptoms to genes that are currently included in our dataset.

In summary, the growing international interest in genomic newborn screening has prompted important questions about which genes and disorders should be considered for inclusion. Due to the substantial variation in the genes included by 27 NBSeq programs, we developed an evidence-based approach to considering gene selection that draws from a comprehensive data repository encompassing over 4,000 genes. Rather than creating a static list of genes for universal implementation, our dynamic ranking system is adaptable and can be updated as new knowledge about genes, disorders, and therapeutics emerges. This work will support gene selection for both research and public health programs considering the use of population-wide NBSeq.

Data Availability

All datasets generated and/or analyzed in this study, along with the Stata, R, and Python code necessary to replicate the results, are available at https://github.com/tjminten/gim_genelist.

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Ethics Declaration

This study did not involve experiments on human participants or animals. All outcome data utilized in this research were aggregated and obtained from previously published sources, which are cited within the manuscript. As no individualized or patient-specific data were used, Institutional Review Board (IRB) or Research Ethics Committee (REC) approval were not required.

Conflict of Interest

L.M.A., A.J.C. and R.J.T. are employees and shareholders at Illumina Inc. N.G. is co-founder and equity owner of Datavisyn. N.B.G. provides occasional consulting services to RCG

Consulting and receives honoraria from Ambry Genetics. R.C.G. has received compensation for advising the following companies: Allelica, Atria, Fabric, Genomic Life and Juniper Genomics; and is co-founder of Genome Medical and Nurture Genomics. B.E.R. and K.L.S. are consultants at Nurture Genomics. L.S. received personal compensation from Zentech and Illumina Inc. P.T. is a co-founder of PlumCare RWE, LLC.

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Figure Legends

Figure 1. Research and commercial genomic newborn screening (NBSeq) programs. Gene lists from 27 of these programs were included in the analysis (denoted with an asterisk). Intended enrollment sizes are indicated where available.

Figure 2. Description, concordance and content of gene lists of genomic newborn screening programs.

A. Clinical areas of 4,299 genes included in BabySeq.
B. Counts and clinical areas of genes included in 26 research and commercial genomic newborn screening programs (n=4,390).
C. Jaccard similarity index, which offers a quantitative comparison of how closely related the gene lists are.
D. UpSet plot of gene lists of 4 large research studies. The matrix below the bar graph represents each individual study and their intersections (n=818).
E. Inclusion of genes associated with core Recommended Uniform Screening Panel (RUSP) conditions. The x-axis is each genomic newborn screening program and y-axis are individual genes; the corresponding cell is colored if the gene is included on a given list.
F. Inclusion of genes associated with secondary RUSP conditions.
G. Inclusion of genes on 20 lists or more that are not associated with RUSP conditions.

Figure 3. Determinants and prediction model of gene inclusion in genomic newborn screening. In a and b, RUSP category (n=4,474), survey recommendation and orthogonal test (n=649), evidence base, efficacy, penetrance, disease severity, treatment acceptability and neonatal or infant onset (n=749). ROC, Receiver Operating Characteristic; AUC, Area Under the Curve.

A. Regression coefficients (and confidence intervals) associated with various gene and disease characteristics predicting inclusion across gene lists.
B. Heat map with regression coefficients associated with gene and disease characteristics for each individual genomic newborn screening program.
C. ROC curves for three prediction models in the hold-out test set (n=895 genes).
D. Scatter plot of predicted versus observed gene list inclusion, showing the fit of the boosted trees model on the 20% hold-out set (n=895 genes).

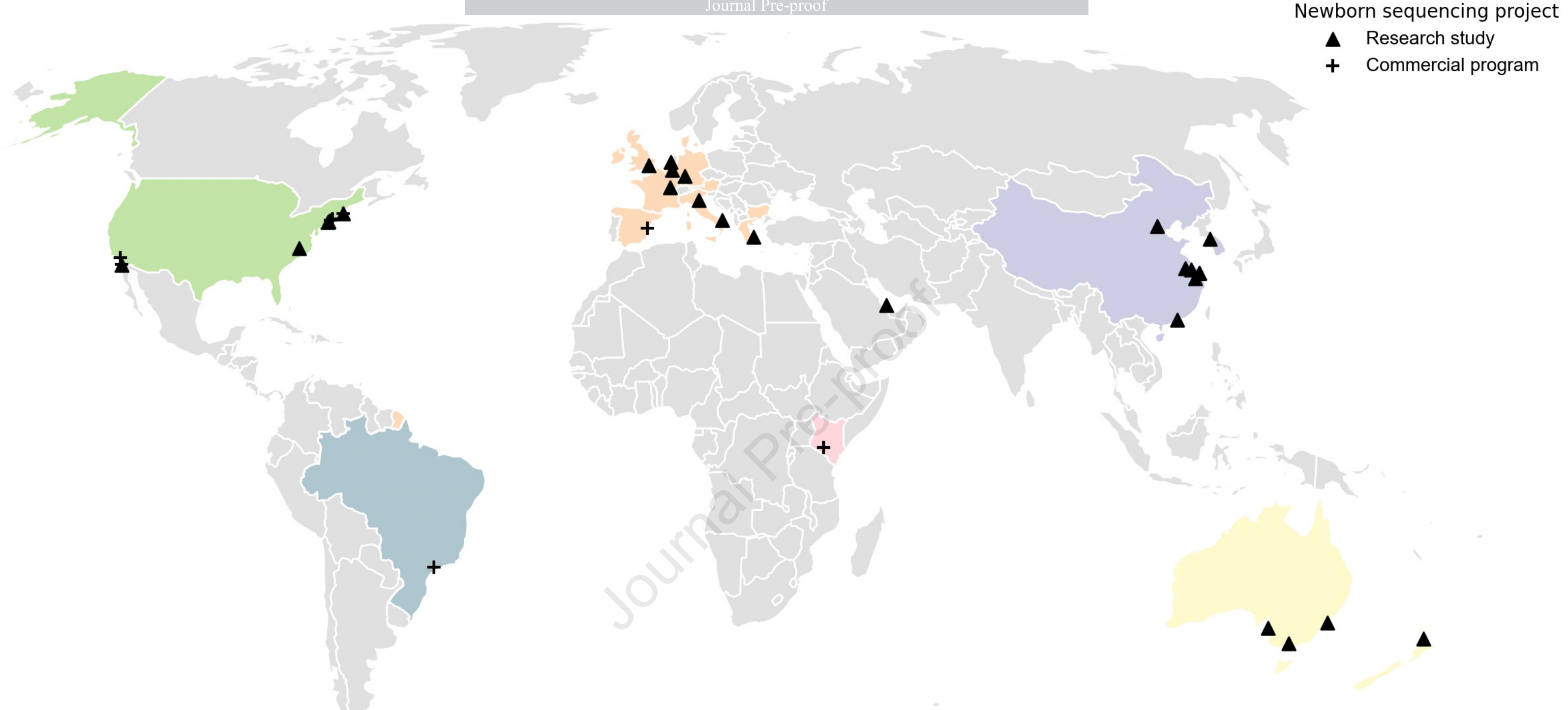
Table 1. Percentage of positive results from genomic newborn screening research programs. Positive cases of *G6PD*, as well as follow-up data for positively screened infants reported where available. PPV, positive predictive value.

		Sequencing data				Follow-up data		
NBSeq research study	Genes	Infants sequenced	G6PD deficiency	Total positive	Total positive (in %)	Total follow-up	Total diagnosed	PPV
BabyDetect	405	3,847	44	71	1.85%			
PerkinElmer Panel	268	606	4	13	2.15%			
Wang et al. 2023	164	10,334		232	2.25%	231	50	22%
gnSTAR	134	4,986	40	113	2.27%			
Chen et al. 2023	142	29,601	689	813	2.75%	797	402	50%
GUARDIAN Study	237	4,000		147	3.68%	151	120	79%
NESTS	465	11,484	338	902	7.85%	414	50	12%
PerkinElmer GS	6,000	562	4	46	8.19%			
NeoExome	601	3,049		271	8.89%			
BabySeq	4,299	159	1	15	9.43%			
TOTAL		**68,628**		**2,623**	**3.82%**			

Table 2. List of 50 genes with highest predicted inclusion across NBSeq programs, excluding genes on the Recommend Uniform Screening Panel (RUSP).
ASQM, Age-Based Semi Quantitative Metric.

Rank	Gene	Disorder	OMIM Phenotype	Clinical area	BabySeq category	ClinGen validity	ClinGen Actionability (/12)	ASQM score (/15)	Expert Recommendation	Observed list inclusion (/27)	Predicted list inclusion
13	*G6PC1*	Glycogen storage disease Ia	232200	Metabolism	A			13	93%	26	96%
17	*OTC*	Ornithine transcarbamylase deficiency	311250	Metabolism	A	Definitive	10AB	14	98%	26	96%
40	*SLC37A4*	Glycogen storage disease Ib	232220	Metabolism	A			13	93%	25	91%
41	*CPS1*	Carbamoylphosphate synthetase I deficiency	237300	Metabolism	A	Definitive	9AB	13	86%	25	90%
47	*SLC25A15*	Hyperornithinemia-hyperammonemia-homocitrullinemia syndrome	238970	Metabolism	A	Definitive		10	85%	25	88%
51	*AGL*	Glycogen storage disease IIIa	232400	Metabolism	A	Definitive		12	85%	24	86%
52	*NAGS*	N-acetylglutamate synthase deficiency	237310	Metabolism	A	Definitive	10NC	14	85%	22	86%
55	*ALPL*	Hypophosphatasia, infantile	241500	Endocrinology	A	Definitive	10CC	11	71%	23	84%
56	*GALNS*	Mucopolysaccharidosis IVA	253000	Metabolism	A	Definitive	9CA	9	87%	23	84%
57	*ALDOB*	Fructose intolerance, hereditary	229600	Metabolism	A		10NC	14	84%	23	83%
60	*BTK*	Agammaglobulinemia, X-linked 1	300755	Immunology	A	Definitive			81%	24	82%
65	*F9*	Hemophilia B	306900	Hematology	A	Definitive	9CB	14	90%	23	81%
68	*CYBB*	Chronic granulomatous disease, X-linked	306400	Immunology	A			12	74%	25	80%
69	*G6PD*	Hemolytic anemia, G6PD deficient (favism)	300908	Hematology	A	Definitive	7DC	12	80%	23	80%
73	*CYP11B1*	Adrenal hyperplasia, congenital, due to 11-beta-hydroxylase deficiency	202010	Endocrinology	A			11	92%	21	79%
74	*SMPD1*	Niemann-Pick disease	257200	Metabolism	A	Definitive		9	85%	21	78%
75	*ARSB*	Mucopolysaccharidosis type VI (Maroteaux-Lamy)	253200	Metabolism	A	Definitive		11	92%	22	77%
76	*CTNS*	Cystinosis, nephropathic	219800	Nephrology	A	Definitive	9CB	14	80%	20	77%
77	*ATP7B*	Wilson disease	277900	Metabolism	A	Definitive	11CA	12	81%	21	77%
78	*CYBA*	Chronic granulomatous disease 4, autosomal recessive	233690	Immunology	A			12	74%	18	77%
79	*ABCC8*	Hyperinsulinemic hypoglycemia, familial, 1	256450	Endocrinology	A	Definitive		14	81%	20	75%
80	*RB1*	Retinoblastoma	180200	Oncology	A	Definitive	10CB	13	89%	21	75%
81	*ALDH7A1*	Epilepsy, early-onset, 4, vitamin B6-dependent	266100	Neurology		Definitive	11CB	12	86%	21	74%
82	*TG*	Thyroid dyshormonogenesis 3	274700	Endocrinology	A			14		18	73%
84	*TPO*	Thyroid dyshormonogenesis 2A	274500	Endocrinology	A			14		18	71%
85	*PHEX*	Hypophosphatemic rickets, X-linked dominant	307800	Endocrinology			9CC		74%	20	71%
86	*TH*	Segawa syndrome, recessive	605407	Neurology	A	Definitive		13	70%	20	71%
87	*ATP7A*	Menkes disease	309400	Metabolism	A	Definitive	9CA	12	84%	19	71%
89	*SLC2A1*	GLUT1 deficiency syndrome 1, infantile onset, severe	606777	Metabolism	A	Definitive		11	90%	19	71%
90	*GLA*	Fabry disease	301500	Metabolism	A	Definitive	9CA	12	83%	20	70%
91	*DUOX2*	Thyroid dyshormonogenesis 6	607200	Endocrinology	A			13		20	70%
92	*WAS*	Wiskott-Aldrich syndrome	301000	Immunology	A	Definitive	9CC		68%	20	69%
93	*FBP1*	Fructose-1,6-bisphosphatase deficiency	229700	Metabolism				13	80%	18	69%
94	*PYGL*	Glycogen storage disease VI	232700	Metabolism	A			10	83%	19	69%
96	*SPR*	Dystonia, dopa-responsive, due to sepiapterin reductase deficiency	612716	Neurology	A	Definitive		12	70%	20	69%
97	*CD40LG*	Immunodeficiency, X-linked, with hyper-IgM	308230	Immunology	A	Definitive		11	74%	17	68%
98	*LIPA*	Wolman disease	620151	Metabolism	A	Definitive		7	80%	19	67%
100	*GUSB*	Mucopolysaccharidosis VII	253220	Metabolism	A	Definitive		9	89%	19	67%
101	*SCNN1B*	Pseudohypoaldosteronism, type IB2, autosomal recessive	620125	Endocrinology	A			14	49%	17	66%
102	*POU1F1*	Pituitary hormone deficiency, combined or isolated, 1	613038	Endocrinology	A			12	66%	18	66%
103	*TSHR*	Hypothyroidism, congenital, nongoitrous, 1	275200	Endocrinology	A			12		24	66%
104	*AVPR2*	Diabetes insipidus, nephrogenic, 1	304800	Endocrinology	A			11	60%	18	66%
105	*KCNJ11*	Diabetes mellitus, transient neonatal 3	610582	Endocrinology	A	Definitive		12	76%	18	64%
106	*GATM*	Cerebral creatine deficiency syndrome 3	612718	Metabolism		Definitive		10	85%	17	64%

108	*GLUD1*	Hyperinsulinism-hyperammonemia syndrome	606762	Metabolism	A	Definitive	11	87%	18	63%
110	*PHKB*	Phosphorylase kinase deficiency of liver and muscle, autosomal recessive	261750	Metabolism	A	Definitive	13	76%	17	63%
111	*CYP17A1*	17,20-lyase deficiency, isolated	202110	Endocrinology				90%	18	62%
114	*NPC1*	Niemann-Pick disease	257220	Metabolism	A	Definitive	12	69%	19	62%
115	*SLC7A7*	Lysinuric protein intolerance	222700	Metabolism	A	Definitive	12	85%	16	62%
116	*PROP1*	Pituitary hormone deficiency, combined, 2	262600	Endocrinology	A		13	66%	19	62%



North America

- ▲ BabySeq* (MA), 100,000
- ▲ BeginNGS* (CA), 2,000
- ▲ Early Check* (NC), 10,000
- ▲ GUARDIAN* (NY), 100,000
- ▲ ScreenPlus (NY)
- + FORESITE 360* (CA)
- + Fulgent* (CA)
- + Nurture Genomics* (MA)
- + PerkinElmer* (CT)
- + Sema4* (CT)

Europe

- ▲ BabyDetect* (BEL), 40,000
- ▲ FirstSteps* (GRE), 101,000
- ▲ Generation* (ENG), 100,000
- ▲ Screen4Care* (EU), 18,000
- ▲ Genoma Puglia* (ITA), 6,000
- ▲ Perigenomed (FRA), 20,000
- ▲ Cradle (NED)
- ▲ New_Lives (GER)
- + Igenomix* (ESP)

Asia

- ▲ Chen et al.* (CHI), 29,601
- ▲ gnSTAR* (CHI), 4,986
- ▲ Jian et al.* (CHI), 321
- ▲ Lee et al.* (KOR), 48
- ▲ Luo et al.* (CHI), 1,127
- ▲ NeoExome* (CHI), 3,423
- ▲ NeoSeq* (CHI), 196
- ▲ NESTS* (CHI), 11,484
- ▲ Wang et al.* (CHI), 10,224
- ▲ Sidra Medicine (QAT)

South America

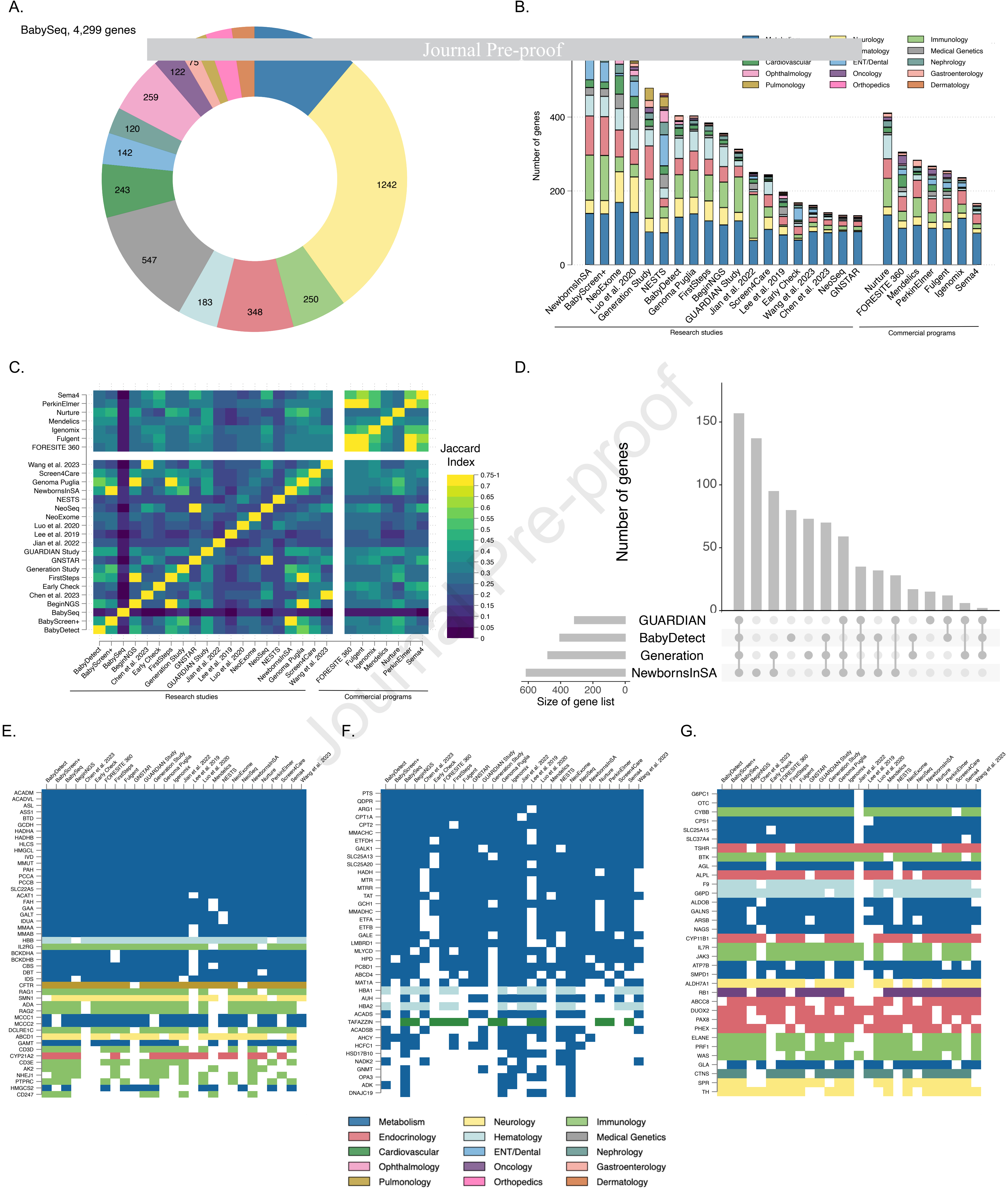
- + Mendelics* (BRA)

Africa

- + KIBs (KEN)

Oceania

- ▲ BabyScreen+* (AUS), 1,000
- ▲ NewbornsInSA* (AUS), 1,000
- ▲ Newborn Gen Prog (NZ)
- ▲ TRAIL (AUS)


A.
BabySeq, 4,299 genes
1242
547
348
250
183
243
142
120
259
122
75
B.
Number of genes
0
200
400
NewbornsInSA
BabyScreen+
NeoExome
Luo et al. 2020
Generation Study
NESTS
BabyDetect
Genoma Puglia
FirstSteps
BeginNGS
GUARDIAN Study
Jian et al. 2022
Screen4Care
Lee et al. 2019
Early Check
Wang et al. 2023
Chen et al. 2023
NeoSeq
GNSTAR
Nurture
FORESITE 360
Mendelics
PerkinElmer
Fulgent
Igenomix
Sema4
Research studies
Commercial programs
Metabolism
Neurology
Immunology
Hematology
Medical Genetics
Cardiovascular
ENT/Dental
Nephrology
Ophthalmology
Oncology
Gastroenterology
Pulmonology
Orthopedics
Dermatology
C.
Sema4
PerkinElmer
Nurture
Mendelics
Igenomix
Fulgent
FORESITE 360
Wang et al. 2023
Screen4Care
Genoma Puglia
NewbornsInSA
NESTS
NeoSeq
NeoExome
Luo et al. 2020
Lee et al. 2019
Jian et al. 2022
GUARDIAN Study
GNSTAR
Generation Study
FirstSteps
Early Check
Chen et al. 2023
BeginNGS
BabySeq
BabyScreen+
BabyDetect
Jaccard Index
0.75-1
0.7
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Research studies
Commercial programs
D.
Number of genes
0
50
100
150
GUARDIAN
BabyDetect
Generation
NewbornsInSA
600
400
200
0
Size of gene list
E.
ACADM
ACADVL
ASL
ASS1
BTD
GCDH
HADHA
HADHB
HLCS
HMGCL
IVD
MMUT
PAH
PCCA
PCCB
SLC22A5
ACAT1
FAH
GAA
GALT
IDUA
MMAA
MMAB
HBB
IL2RG
BCKDHA
BCKDHB
CBS
DBT
IDS
CFTR
RAG1
SMN1
ADA
RAG2
MCCC1
MCCC2
DCLRE1C
ABCD1
GAMT
CD3D
CYP21A2
CD3E
AK2
NHEJ1
PTPRC
HMGCS2
CD247
F.
PTS
QDPR
ARG1
CPT1A
CPT2
MMACHC
ETFDH
GALK1
SLC25A13
SLC25A20
HADH
MTR
MTRR
TAT
GCH1
MMADHC
ETFA
ETFB
GALE
LMBRD1
MLYCD
HPD
PCBD1
ABCD4
MAT1A
HBA1
AUH
HBA2
ACADS
TAFAZZIN
ACADSB
AHCY
HCFC1
HSD17B10
NADK2
GNMT
OPA3
ADK
DNAJC19
G.
G6PC1
OTC
CYBB
CPS1
SLC25A15
SLC37A4
TSHR
BTK
AGL
ALPL
F9
G6PD
ALDOB
GALNS
ARSB
NAGS
CYP11B1
IL7R
JAK3
ATP7B
SMPD1
ALDH7A1
RB1
ABCC8
DUOX2
PAX8
PHEX
ELANE
PRF1
WAS
GLA
CTNS
SPR
TH
Metabolism
Neurology
Immunology
Endocrinology
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ENT/Dental
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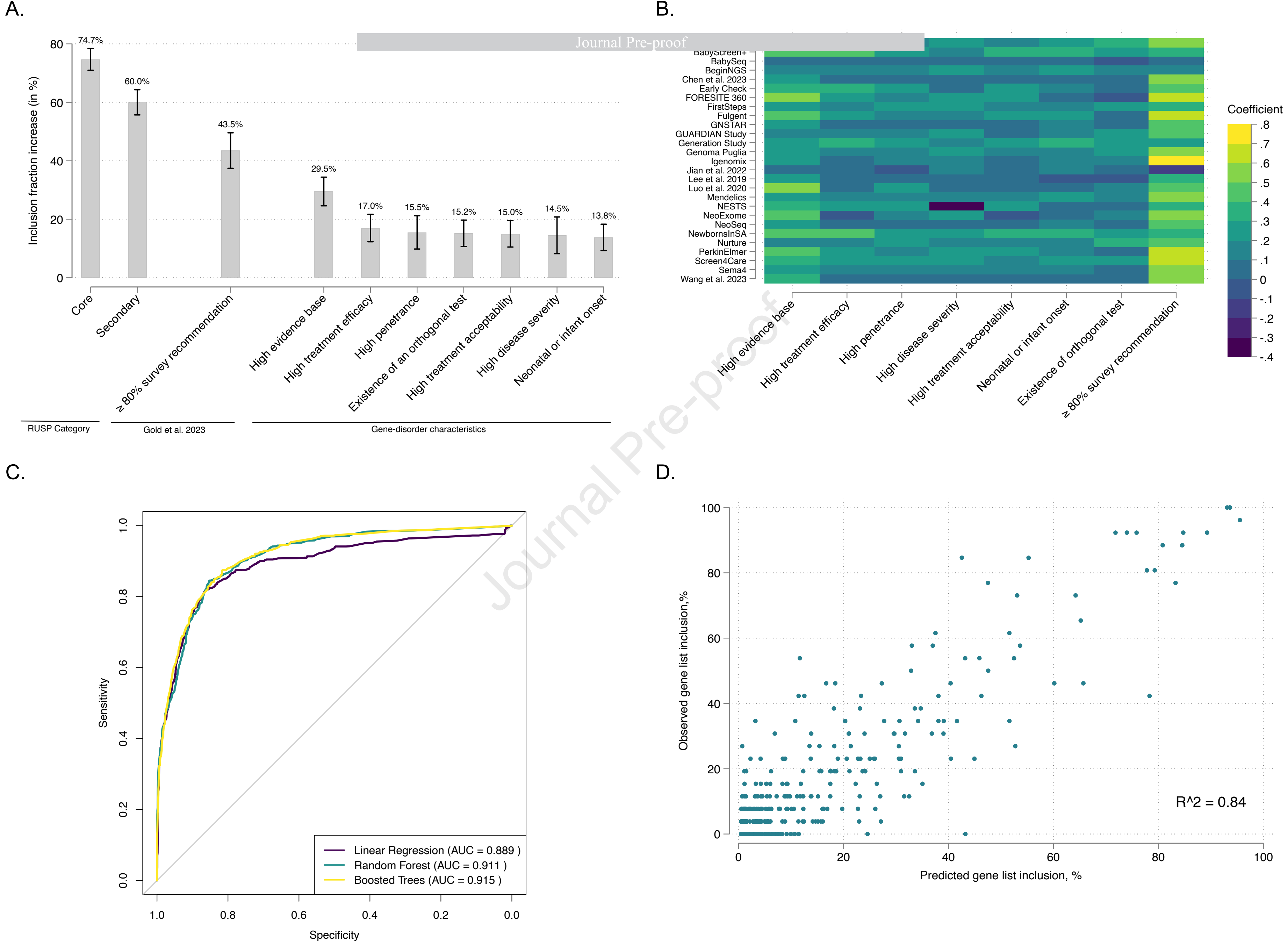
Gastroenterology
Pulmonology
Orthopedics
Dermatology


A.
Inclusion fraction increase (in %)
80
60
40
20
0
74.7%
60.0%
43.5%
29.5%
17.0%
15.5%
15.2%
15.0%
14.5%
13.8%
Core
Secondary
≥ 80% survey recommendation
High evidence base
High treatment efficacy
High penetrance
Existence of an orthogonal test
High treatment acceptability
High disease severity
Neonatal or infant onset
RUSP Category
Gold et al. 2023
Gene-disorder characteristics
B.
BabyScreen+
BabySeq
BeginNGS
Chen et al. 2023
Early Check
FORESITE 360
FirstSteps
Fulgent
GNSTAR
GUARDIAN Study
Generation Study
Genoma Puglia
Igenomix
Jian et al. 2022
Lee et al. 2019
Luo et al. 2020
Mendelics
NESTS
NeoExome
NeoSeq
NewbornsInSA
Nurture
PerkinElmer
Screen4Care
Sema4
Wang et al. 2023
High evidence base
High treatment efficacy
High penetrance
High disease severity
High treatment acceptability
Neonatal or infant onset
Existence of orthogonal test
≥ 80% survey recommendation
Coefficient
.8
.7
.6
.5
.4
.3
.2
.1
0
-.1
-.2
-.3
-.4
C.
Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
Specificity
1.0
0.8
0.6
0.4
0.2
0.0
Linear Regression (AUC = 0.889)
Random Forest (AUC = 0.911)
Boosted Trees (AUC = 0.915)
D.
Observed gene list inclusion,%
0
20
40
60
80
100
Predicted gene list inclusion, %
0
20
40
60
80
100
R^2 = 0.84