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RESEARCH ARTICLE

Genome-to-Treatment and Begin Newborn Genomic Screening: A Review of System Guides for the Acute Management and Newborn Screening Follow-up of Genetic Disorders in Infants and Children

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ABSTRACT

Many rare genetic conditions have effective interventions; however, without timely implementation, these conditions can progress to severe morbidity and even mortality. For the most relentlessly progressive conditions, a rapid molecular diagnosis alone is not sufficient to improve outcomes in these conditions. Frontline physicians are frequently unfamiliar with many of these conditions and their treatments. Additionally, the field of genetics is rapidly expanding and new conditions along with new interventions are being increasingly described. Here we present a review of the development and use of two linked automated systems developed to help overcome this problem. The first, GTR X (gene to treatment) has been developed to assist with the management of the acutely ill neonate infant or child in the neonatal or pediatric intensive care unit found to have a recognized genetic disease. Subsequently with expansion of newborn screening to potentially involve rapid next generation sequencing of newborns in parallel with the current analyte based newborn screening, we have developed an additional system, BeginNGS, intended to be used with currently available ACTion (ACT) sheets that will address both recommended confirmatory testing and initial interventions, particularly focusing on conditions that are not amenable to conventional newborn screening.

Introduction

The approximately 7200 known genetic disorders cause morbidity and mortality in a sizable proportion of the pediatric population[1-7]. Many of these conditions are first recognized in the neonatal, pediatric or cardiovascular ICU and frontline physicians are often unfamiliar with many of these conditions and their treatments[8-10]. Of the approximately 140 million children worldwide who are affected by rare genetic diseases, it is estimated that about 30% will not survive to their 5th birthdays[12,13]. In the ICU setting, progression of these genetic diseases is often extremely rapid resulting in morbidity and/or early death without timely diagnosis and treatment. For the ICU, the initial technological solution to this problem was rapid diagnostic whole genome sequencing. This enabled concomitant diagnostic evaluation of almost all genetic diseases, often in less than 24 hours[14-19]. In fact, it has become apparent that available evidence does not support limiting the use of rapid or ultra rapid exome or genome sequencing to cases of predicted high diagnostic yield in the intensive care unit[20]. However, it is apparent that frontline care providers often lack familiarity with these diseases so outcomes may not be improved[21-25]. It is also clear that rapid sequencing and genetic diagnosis are crucial parts of inpatient management. These should be accessible not only to academic medical centers but also to hospitals in the community setting[26]. Geographic distance to specialty centers correlates with time to diagnosis, receipt of specialty care, and outcomes[27-29]. Genomic technologies, however, have outpaced medical education. At issue is practitioners' knowledge about this testing and its most appropriate application. Several recent studies[21-25] have addressed the question of provider comfort and attitude towards genetic testing. Most acknowledge a positive attitude toward genetic testing but lack confidence in test result interpretation and in application of these results to disease treatment. Many are unaware of resources that can be used to direct management[21-25]. Primary care providers clearly recognize the need to integrate genetics into their practices, but have identified several barriers to doing so, including lack of knowledge and confidence along with access to timely formal and informal specialist consultation[30]. GTRx (Gene-to- Treatment) and BeginNGS (Begin Newborn Genomic Screening), two web-based information systems for either acute management or follow-up of abnormal molecular newborn screening results, easily fulfill the requirement of providing "accessible just in time support" as the clinician is reaching out to appropriate consultants[29, 31].

We are now at a crossroads with newborn screening as well. Current newborn screening is based primarily on analyte testing with second tier confirmation of results. Some conditions do require initial molecular testing, for example, spinal muscular atrophy, cystic fibrosis, and Krabbe disease, but these do not represent the majority of conditions currently identified by NBS. There has been much interest in pursuing first tier molecular analysis for newborn screening[32-36]. Ideally, this would not replace current newborn screening modalities but would function as an adjunct. It would also allow for diagnosis of conditions for which no analyte testing is currently available, but for which early intervention clearly has the potential of improving

outcomes. While the initial intent was to generate a resource that linked phenotype to genotype, it has become increasingly clear that analysis might prove more useful if genotype is then linked to phenotype. The ultimate goal is to provide care for affected children in a timely fashion that has the potential to improve long term outcomes.

The aim and scope of this article is to review the current status of two web-based information resources for practitioners on the front line for critically ill children as well as expanding this resource for use with outpatient molecular testing and molecular newborn screening results.

Gene-to-Treatment

As noted previously, many genetic diseases have either only recently been discovered or are ultra rare and therefore no evidence-based treatment guidelines have been developed. Management strategies often require literature reviews and may be interspersed in the form of case reports, case series or cohort studies. Relative efficacy may not be obvious. Information resources pertaining to management are not complete and are not typically targeted toward acute ICU treatment or frontline physicians[37]. In fact, the majority of the current literature about digital support systems focuses on the use of the electronic medical record which tends to focus on more common conditions[38,39]. Thus, when a frontline physician receives a molecular diagnosis for a condition for which little information is available, they may experience an unsupportable burden to search and synthesize the available treatment evidence for these rare genetic conditions[29]. Recently, *Bick et al.*[40] have generated an on-line tool which serves as a compilation of monogenic diseases for which there are treatments that are directed against the mechanism of disease. This website contains a lot of information in outline form, including references to current treatment, links to guidelines and other information (Rx-genes.com (https://www.rx-genes.com/)[41] The major difference between Rx-genes.com and GTRx (https://radygenomics.org/gtrx-genome-to-treatment/) is that the former is a more general source, while the latter has been designed to provide information for use in the acute setting.

The development of GTRx and initial utility have previously been described[29]. Briefly, the development of GTRx occurred in eight phases (Figure 1). In phase one, treatable disorders were identified, literature was reviewed, and clinician examination and whole genome sequencing experience was evaluated. Phase two involved integration of information resources. Phase three centered on development of the GTRx web resource. Phase four in the initial iteration of GTRx utilized artificial intelligence and expert curation of the treatment literature (drugs, devices, diets, surgeries and other interventions) that could be implemented. This was followed, in phase 5, by independent review of indications, contraindications, efficacy and evidence of insufficiency of each treatment in each disorder by experts in clinical, pediatric and biochemical genetics/metabolism. An initial pilot of 15 gene disease pairs was undertaken, after which an additional examination was pursued of 358 genes associated with 563 genetic conditions, representing 8% of 7103 single locus genetic diseases that met the following

criteria: (1) conditions that presented acutely in childhood that would likely result in admission to a neonatal, pediatric or cardiovascular intensive care unit, (2) conditions had somewhat effective treatments or acute interventions, (3) conditions had a high probability of rapid progression or irreversible sequelae without treatment and (4) conditions were diagnosable by rapid whole genome sequencing. Several assumptions were made, particularly around the minimal data elements needed by providers after receiving a rapid Whole Genome Sequencing (rWGS) result. In the setting of a newly diagnosed genetic disease in a critically ill child, they needed to know the indicated interventions, optimal time to administration, efficacy, evidence for efficacy, contraindications, and natural history without treatment (Box 1)[42]. It was also assumed that adequate resources existed to provide guidance about drug dosing, frequency, route of administration, drug-drug interactions or labeled contraindications. The disease management guidance system (GTRx- Gene to Treatment) needed to be authoritative and consensus-driven. For each genetic disease, the full texts of all MEDLINE/PubMed references that mentioned a drug, device, diet or surgery used to treat the disease using three artificial intelligence based search engines (Mastermind, Genomenon; Rancho Biosciences, Epam Systems, Figure 2)[43] were indexed. The resultant datasets were manually curated for relevance and specificity, and to extract the required data elements. The manually curated datasets and links to the information resource were integrated into a custom Research Electronic Data Capture (REDCap) survey for expert review (Figure 2)[29,44]. Minimal structured data elements were identified that were required for FAIR-compliant systemic literary reviews to create a virtual acute management system for clinicians[42].

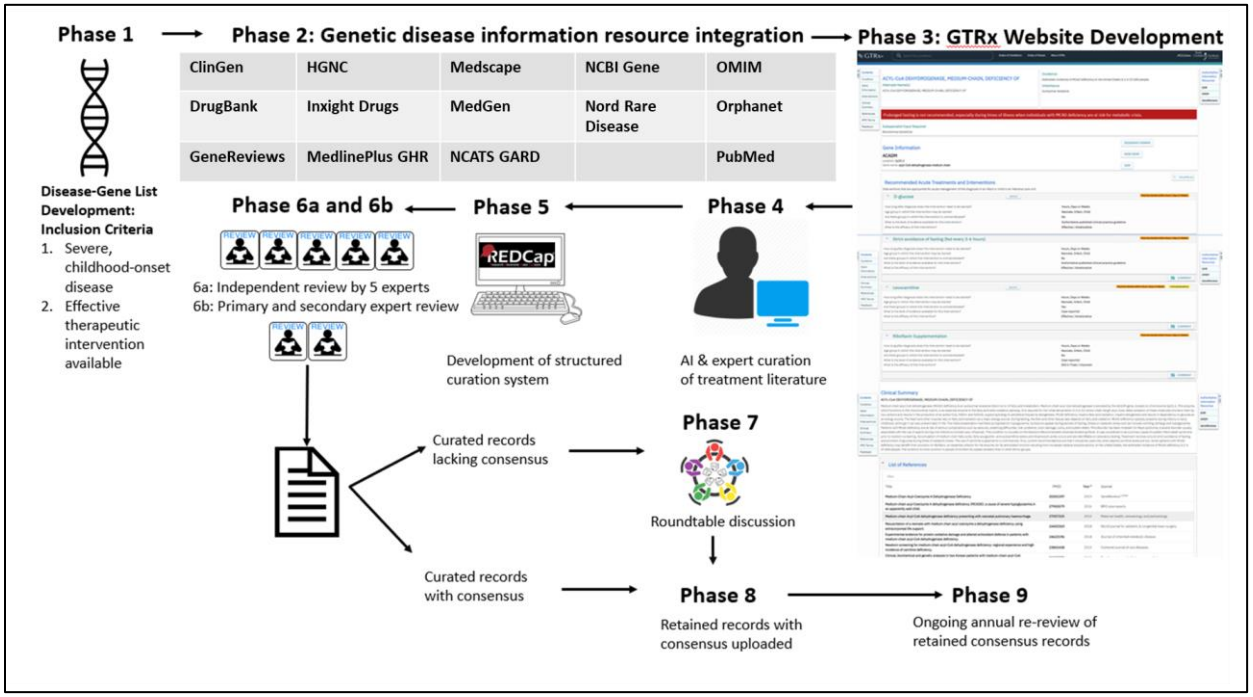

Figure 1: Flowchart of the development of Genome-To-Treatment (GTRx), a virtual system for acute management guidance for rare genetic diseases. Phase 1- Compilation of a comprehensive gene-genetic disease list for severe, childhood onset conditions with available established treatment, Phase 2, integration of 13 information sources relating to rare genetic diseases. Phase 3-Development of GTRx web resource with integrated information resources. Phase 4- Automated artificial intelligence-based searching and manual curation of published evidence of treatments. Phase 5- Development of custom REDCap system for structured assessment of genes, disorders, and interventions. Phase 6a-Independent manual review of curated interventions for first 15 pilot genes-disease pairs by five experts. Phase6b- Primary and secondary reviews of remaining gene disease pairs. Phase 7- Round table discussion of records lacking consensus. Phase 8- Upload of retained consensus records to GTRx web source. Phase 9- Ongoing annual review of retained consensus records[29].

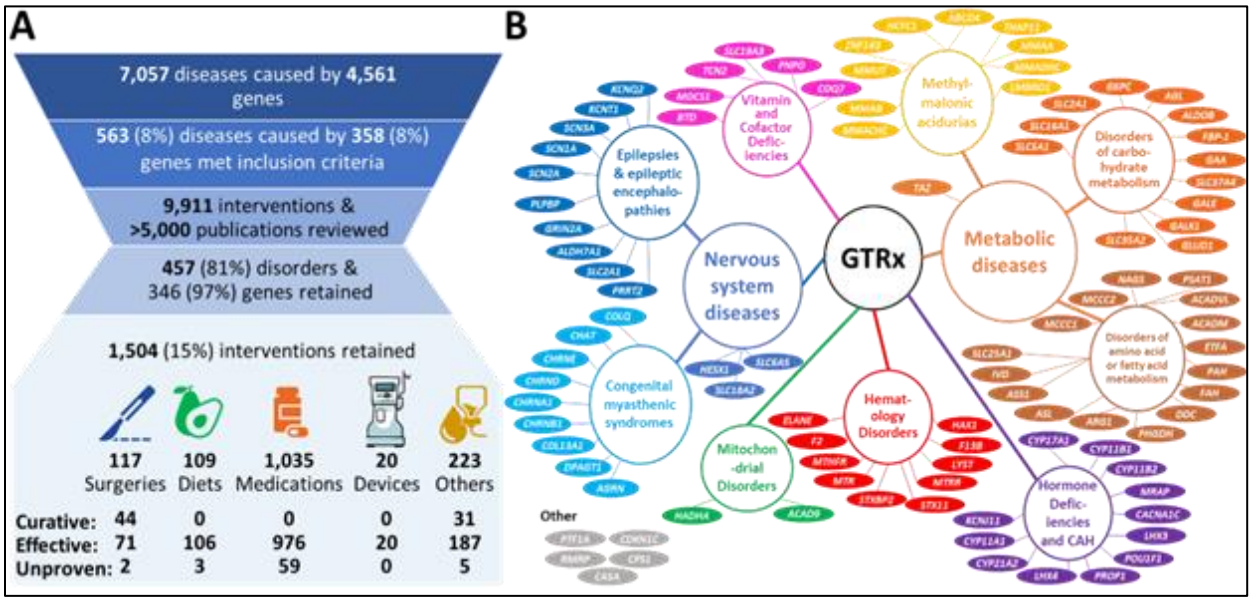

Figure 2: GTRx disease, gene and literature filtering, and final content. A: A modified PRISMA flowchart showing filtering steps and summarizing results of review of 563 unique disease-gene dyads. B. Genetic disease types and disease genes feature in the first 100 GTRx genes[29].

Box 1 Minimal structured data elements for FAIR-compliant systemic literary reviews to create a virtual acute management support system for clinicians[29]

- Disease, gene, incidence, inheritance mode(s)
- Defined appropriate subspecialist consultant(s)
- Clinical summary and natural history of disease
- Set of appropriate acute treatments
- For each treatment
  - Efficacy
    - Curative
    - Effective/Ameliorative
    - Still in trials
    - Contraindicated
  - Evidence supporting efficacy
    - Authoritative published guidelines
    - Cohort study or studies
    - Case reports
    - Expert opinion
  - Optimal timeframe to initiate after diagnosis
    - Hours
    - Days/Weeks
    - Years
  - Appropriate age group(s)
    - Neonates (first 4 weeks)
    - Infants (1 month through 12 months)
    - Children (1 year-21 years)
  - Contraindicated groups
  - Banner warning (if any)

After completion of the initial pilot phase examining 15 conditions, which was intended to test the process (each disease and intervention was reviewed by all five geneticists highly experienced in clinical, pediatric and biochemical genetics), 2 reviewers independently appraised data for each gene/disease association followed by group discussion to resolve any discrepancies. If reviewers did not reach consensus on inclusion of conditions or interventions, phase seven involved a roundtable discussion amongst all reviewers in order to reach consensus. Phase 8 was completed after uploading retained consensus records to the GTRx web resource. In this phase of development, consensus was reached for 189/190 treatments during the pilot review process with the number of interventions evaluated per gene ranging from 1-140. Of 8298 interventions reviewed, 1242 (15%) were retained and of 388 diseases reviewed, 364, (93.8%) were retained. From the perspective of the reviewers, automated data retrieval was most effective for single gene/disease associations. Artificial intelligence did not differentiate literature to support disease specific management from literature with

interventions listed, for example, as part of clinical history. This resulted in many non-disease-specific interventions requiring review, particularly for disorders with significant locus heterogeneity. Multiple interventions could be consolidated as a single intervention by drug class or mode of effect, allowing for provider and hospital autonomy in specific management of conditions. Finally, disorders for which there is no effective intervention or for which the disease management would not be relevant in the ICU context were removed leaving only effective or curative interventions with the potential to significantly affect outcomes. Notably absent from the first version of GTRx was confirmatory testing.

The clinical utility, ease of use and ease of comprehension of the GTRx information resource and management guidance was evaluated by nine senior neonatologists and pediatric intensivists who were not involved in its design or development[29]. On a 10-point Likert scale, their median perception as to whether they would use GTRx was 9, ease of use was 9, and the utility of the information was 6. GTRx was perceived to meet clinical needs somewhat well. In response to specific feedback, the GTRx website was modified to increase ease of use, clarity, and to elicit ongoing feedback.

Several modifications have been made to the initial version of GTRx, the first of which involved identification of confirmatory testing to supplement sequence verification, if applicable. To broaden the applicability of this tool, more generally available treatments have been included in addition to initially identified cutting edge treatments. The recognition that this tool might be useful for the nonemergent management in settings other than the ICU, resulted in inclusion of additional information as well as a gene/condition descriptive summary.

Recognizing that treatments are now evolving at a rapid pace, re-review of already included conditions is occurring annually. We are also planning to add 50 conditions every 6-12 months, each undergoing the same rigorous analysis as was performed for the initial set of conditions, We are soliciting nominations for genes/conditions to be included for review (https://redcap.radygenomiclab.com/surveys/?s=HWYACARFCRK4YHKW). Another aspect that is under annual review pertains to the naming of conditions: efforts are being made, in a manner similar to those being undertaken for OMIM and GeneReviews, to rename previously separate syndromes into a single condition that presents as a spectrum of disease (Table 1).

Table 1: Conditions reclassified as spectra rather than individual conditions for GTRx/BeginNGS (NEG-Negative; POS-Positive)

Record ID	Original Entry	Gene	Modified GTRx/BeginNGS Description
186-ORPHA:39041 186-OMIM:102700	OMENN SYNDROME SEVERE COMBINED IMMUNODEFICIENCY, AUTOSOMAL RECESSIVE, TCELL-NEG, B CELL-NEG, NK CELL-NEG, DUE TO ADENOSINE DEAMINASE DEFICIENCY	ADA	SEVERE COMBINED IMMUNODEFICIENCY, AUTOSOMAL RECESSIVE, T CELL-NEG, B CELL-NEG, NK CELL-NEG, DUE TO ADENOSINE DEAMINASE DEFICIENCY
438-OMIM:241510 438-OMIM:241500	HYPOPHOSPHATASIA, CHILDHOOD HYPOPHOSPHATASIA, INFANTILE	ALPL	HYPOPHOSPHATASIA
735-OMIM:228000 735-OMIM:159950	FARBER LIPOGRANULOMATOSIS SPINAL MUSCULAR ATROPHY WITH PROGRESSIVE MYOCLONIC EPILEPSY	ASAH1	ASAH1-RELATED DISORDER
17642-OMIM:602450 17642-OMIM:603554	SEVERE COMBINED IMMUNODEFICIENCY WITH SENSITIVITY TO IONIZING RADIATION OMENN SYNDROME	DCLRE1C	OMENN SYNDROME
4177-OMIM:230800 4177-OMIM:230900 4177-OMIM:231000 4177-OMIM:231005	GAUCHER DISEASE TYPE I GAUCHER DISEASE TYPE II GAUCHER DISEASE TYPE II GAUCHER DISEASE TYPE IIIC	GBA1	GAUCHER DISEASE
4823-OMIM:604131 4823-OMIM:613978	ALPHA-THALASSEMIA HEMOGLOBIN H DISEASE	HBA1	ALPHA-THALASSEMIA/HEMOGLOBIN H DISEASE
5391-OMIM:607014 5391-OMIM:607015 5391-OMIM:607016	HURLER SYNDROME HURLER-SCHEIE SYNDROME SCHEIE SYNDROME	IDUA	MUCOPOLYSACCHARIDOSIS I
6010-OMIM:312863 6010-ORPHA:39041	COMBINED IMMUNODEFICIENCY, X-LINKED OMENN SYNDROME	IL2RG	COMBINED IMMUNODEFICIENCY, X-LINKED
7530-OMIM:610377 7530-OMIM:260920	MEVALONIC ACIDURIA HYPER-IgD SYNDROME	MVK	MEVALONIC ACIDURIA/HYPER-IgD SYNDROME
30500-OMIM:602066 30500-OMIM:128200 30500-ORPHA:569 30500-OMIM:605751	CONVULSIONS, FAMILIAL INFANTILE, WITH PAROXYSMAL CHOREOATHETOSIS EPISODIC KINESIGENIC DYSKINESIA 1 FAMILIAL OR SPORADIC HEMIPLEGIC MIGRAINE SEIZURES, BENIGN FAMILIAL INFANTILE, 2	PRRT2	PRRT2-ASSOCIATED PAROXYSMAL MOVEMENT DISORDERS
9831-OMIM:609889 9831-OMIM:233650 9831-OMIM:603554 9831-OMIM:601457	ALPHA/BETA T-CELL LYMPHOPENIA WITH GAMMA/DELTA T-CELL EXPANSION, SEVERE CYTOMEGALOVIRUS INFECTION, AND AUTOIMMUNITY COMBINED CELLULAR AND HUMORAL IMMUNE DEFECTS WITH GRANULOMAS SEVERE COMBINED IMMUNODEFICIENCY, AUTOSOMAL RECESSIVE, T CELL-NEG, B CELL-NEG, NK CELL-POS SEVERE COMBINED IMMUNODEFICIENCY, AUTOSOMAL RECESSIVE, T CELL-NEG, B CELL-NEG, NK CELL-POS	RAG1	SEVERE COMBINED IMMUNODEFICIENCY, AUTOSOMAL RECESSIVE, T CELL-NEG, B CELL-NEG, NK CELL-POS

Record ID	Original Entry	Gene	Modified GTRx/BeginNGS Description
9832-OMIM:233650	COMBINED CELLULAR AND HUMORAL IMMUNE DEFECTS WITH GRANULOMAS	RAG2	SEVERE COMBINED IMMUNODEFICIENCY, AUTOSOMAL RECESSIVE, T CELL-NEGA, B CELL-NEG, NK CELL-POS
9832-OMIM:603554	SEVERE COMBINED IMMUNODEFICIENCY, AUTOSOMAL RECESSIVE, T CELL-NEG, B CELL-NEG, NK CELL-POS		
9832-OMIM:601457	SEVERE COMBINED IMMUNODEFICIENCY, AUTOSOMAL RECESSIVE, T CELL-NEG, B CELL-NEG, NK CELL-POS		
16187-OMIM:211530	BROWN-VIALETTO-VAN LAERE SYNDROME 1	SLC52A3	RIBOFLAVIN TRANSPORTER DEFICIENCY
16187-OMIM:211500	FAZIO-LONDE DISEASE		
11117-OMIM:253300	SPINAL MUSCULAR ATROPHY, TYPE I	SMN1	SPINAL MUSCULAR ATROPHY
11117-OMIM:253550	SPINAL MUSCULAR ATROPHY, TYPE II		
11117-OMIM:253400	SPINAL MUSCULAR ATROPHY, TYPE III		
12731-OMIM:300299	NEUTROPENIA, SEVERE CONGENITAL, X-LINKED	WAS	WISCOTT-ALDRICH SYNDROME
12731-OMIM:313900	THROMBOCYTOPENIA 1		
12731-OMIM:301000	WISKOTT-ALDRICH SYNDROME		

Begin Newborn Genomic Screening - a web-based newborn screening tool

Since GTRx has been under development, more convincing arguments for using WGS as a primary, rather than second tier, test for NBS have been posited[31-36]. This is particularly true for conditions for which no specific analyte testing for NBS in dried blood spots (DBSs) is available, although rapid implementation of treatment has the potential to improve outcomes. By definition, newborn screening tests are used to screen all newborns to identify infants who have serious but potentially treatable conditions. Newborn screening does not diagnose conditions: it identifies infants who require further clinical evaluation and testing.

While NBS can greatly improve health outcomes, the number of genetic disorders screened has not kept pace with genomic or therapeutic innovation[45,46,47,48,49]. Between 2006 and 2022, the number of core disorders that were recommended for NBS DBSs in the United States—the Recommended Uniform Screening Panel (RUSP)—increased from 27 to 35, and the number of affected infants identified increased from 6,439 to 6,466[48,49]. Rapid whole genome sequencing from dried blood spots has not only improved technically but is scalable. The large number of conditions that can be analyzed contributes to its cost effectiveness. Although genomic newborn screening has the potential to do much good, it must be emphasized that implementation will require a nuanced approach to maximize benefit and minimize potential harm[50].

Parental views regarding next-generation sequencing have been reported to be generally positive citing benefits of early intervention, preparedness, child autonomy, and altruism[51]. There have been reported concerns about potential negative effects from early intervention, psychosocial harm and religious beliefs. However, parents were most concerned about quality of life. Several studies have reported on parental perception

of NGS in the setting of cancer, epilepsy and cardiovascular disease. While not specifically related to NBS, parents of children with cancer have described realistic hopes and expectations associated with NGS participation even if there was little likelihood of medical benefit. Parental concerns regarding perceived parental guilt if a germline variant were to be disclosed were also reported[52]. There are many monogenic forms of infantile and childhood epilepsy for which specific and personalized treatments are known. Studies regarding parental views on WGS largely parallel those for childhood cancers, although parents anticipated greater benefits from obtaining genetic results and the impact these results would have on their and their child's life[53].While it is true that a whole family would likely benefit from newborn genomic testing, the conclusions from a systematic review by Downie et al[50] identified that the right of a child to self-determination should take precedence.

Physicians were more wary about genetic results from newborn WGS: The American Heart Association has endorsed Genetic testing for patients with a confirmed or suspected diagnosis of an inherited cardiovascular disease or for individuals at high a priori risk resulting from a previously identified pathogenic variant in their family[24] and for all patients diagnosed with recognized forms of cardiomyopathy. A survey of 131 cardiology practitioners in the United States of whom 107 self-identified as nongenetic practitioners found that many cardiology practitioners were not confident about ordering appropriate cardiogenomics tests and making treatment recommendations based on genetic tests[54]. This concern is certainly magnified given incomplete penetrance in a number of conditions. EuroGentest and the European Society of Genetics have published updated specific recommendations for WGS in diagnostics for rare diseases explicitly recommending that WGS should be used for diagnostic purposes only for genes for which a clear association with the disease has been confirmed and that variant interpretation requires clinical information in standardized terms[55]. The concern about identifying variants of uncertain significance is not a trivial one[56]. Laboratories and clinicians will need to develop a strategy for dealing with uncertain findings. A commitment must be made to minimize these findings, and all parties may need to make adjustments to their processes. Even in cases where both variants are pathogenic or likely pathogenic, practical barriers may exist making confirmation of a screen positive infant more difficult since associated costs may be too high for families to absorb: the child's confirmatory testing and follow up care may be covered by insurance but additional testing of parents may not[56]. The bottom line remains- just as with conventional NBS- rWGS NBS is a screen and will not identify or rule out every case. Future reclassification of uncertain variants can only improve sensitivity over time.

Finally, there is the issue of primary-care-providers' lack of knowledge regarding results from the current method of newborn screening[23]. For each NBS marker(s), there is an ACTion (ACT) sheet that describes the short term actions a health professional should follow in communicating with the family and determining the appropriate steps in the follow-up of the infant that has screened

positive. A 2018 survey of pediatric residents identified that only 25% of residents were aware of ACTion sheets and around 50% were either not sure or not aware of the protocol for follow-up of a positive screen. Even in the case of non-analyte/non-molecular-NBS, such as screening for hearing loss, significant knowledge gaps have been identified regarding the existence of hearing screens, the process of following up on screen-fail infants and management recommendations[57]. Most state public health department NBS programs have defined protocols for the follow-up of abnormal NBS results although these may vary from state to state[58,59,60]. Best practice for follow-up of rWGS, as it is currently being studied, will require follow-up algorithms which need to be incorporated into the study design.

We recently evaluated the suitability of the 457 genetic diseases retained in GTRx for NBS-rWGS by using established criteria[61, 62,63], the same expert panel, electronic data capture system, and modified Delphi methods. The panel comprised five pediatric clinical and biochemical geneticists representing hospitals in four states (Figure 3). They met weekly for 1 year. Each week, prior to meeting, they reviewed a set of disorders in a REDCap electronic data capture system. To reach consensus regarding inclusion of each GTRx disorder in NBS-rWGS, the panel considered six questions: (1) Is the natural history of this disease well understood? This question was particularly important for ultra-rare and recently discovered diseases. (2) Is this disease a significant risk for morbidity and mortality in infants or young children? (3) Is a treatment or intervention available that is effective and accepted? (4) Does early treatment improve outcome? (5) Do the benefits of early intervention clearly outweigh the risks? This question was particularly important for drugs with serious adverse effects and other high-risk interventions. (6) For genes with more than one associated disorder, do their treatments differ, and can they be distinguished by rWGS or other tests? The opinions of other pediatric subspecialists at Rady Children's Hospital were sought if consensus was elusive. Overall, this study demonstrated the feasibility of NBS-rWGS for early treatment of several hundred childhood genetic disorders[31]. To support these analyses, an additional web-based tool that parallels GTRx called BeginNGS is under development[31]. This tool, which is still under development, includes many of the same elements present in GTRx although it is more oriented towards confirmation of newborn screening results and initial management recommendations, rather than acute interventions, since the majority of infants will be asymptomatic. Again, a detailed summary of the condition is included. Furthermore, more conditions have been nominated for inclusion and are currently under review.

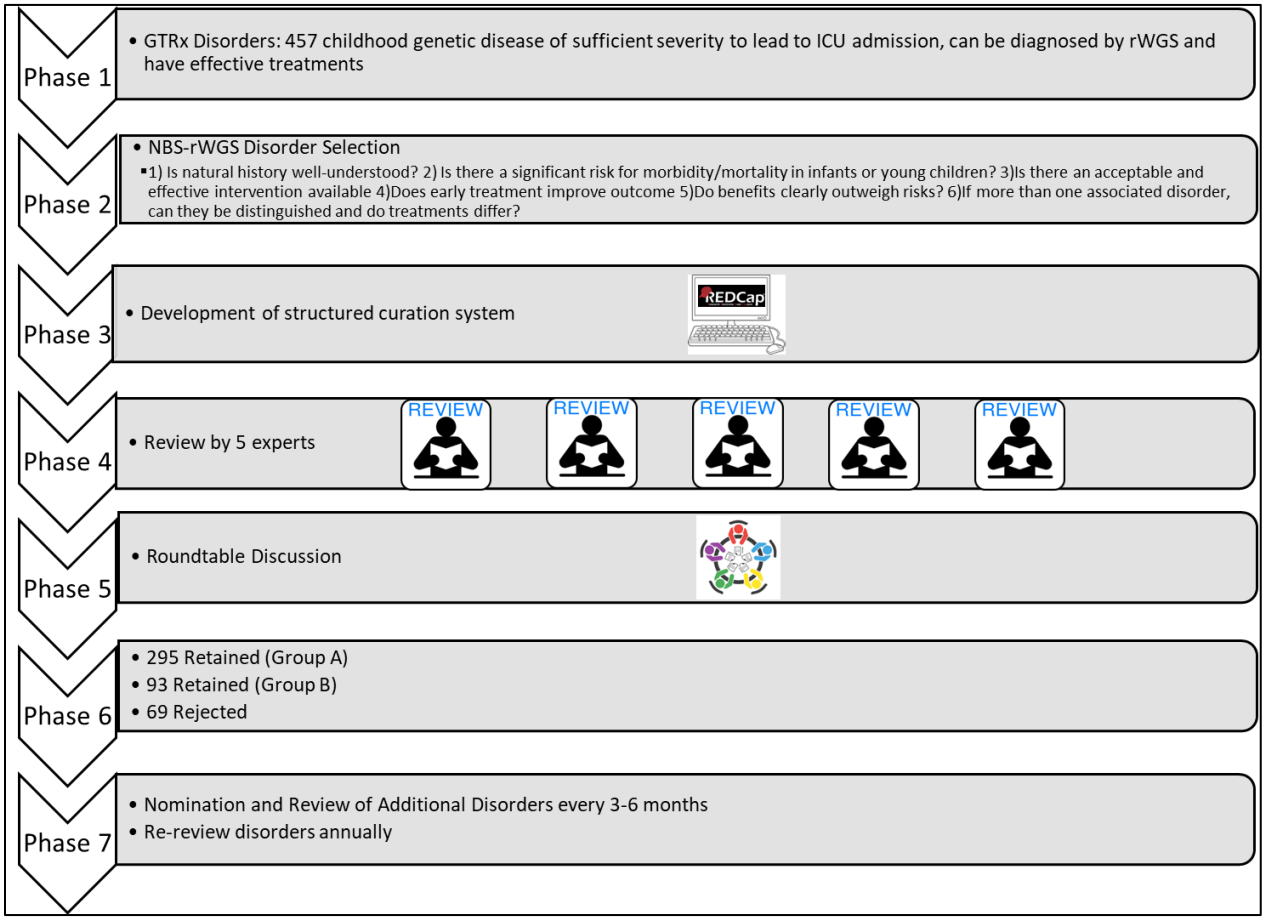

Figure 3: Flowchart of the modified Delphi technique for ongoing selection of disorders for NBS-rWGS after inclusion in the Genome-to-Treatment virtual management guidance system. Abbreviations: ICU, intensive care unit; rWGS, rapid whole-genome sequencing[31]

The process of developing a CLIA-approved test for rWGS NBS using dried blood spots is currently near completion. This test will report only pathogenic and likely pathogenic variants to avoid the confusion and increased false positive rate associated with variants of uncertain significance. Disorders added to BeginNGS were initially divided into Group A and Group B. Group A, disorders are most similar to those that are on the RUSP. Group B disorders are those that do not meet one or more of the Group A criteria, but for which there is a high suspicion that early diagnosis will improve the health and quality of life. The criteria used to select which disorders are on the RUSP are the same as those used to select Group A disorders and are outlined in Box 2.

Box 2 Clinical Criteria Used for Recommendation for Inclusion on the BeginNGS Newborn Screening Panel

Group A criteria:

- The disease shows its first signs and symptoms in infancy or early childhood.
- The progression of the disease process, its signs and symptoms over time, is well understood.
- The gene that causes the disorder is known.
- The majority of people who have mutations in the gene will go on to develop signs and symptoms of the disorder.
- Diagnosis of the disorder will change how an individual is cared for because either there is a specific treatment or by allowing doctors to know the best way to take care of that individual.
- Early diagnosis is known to improve the health and quality of life for the individual.

Group B criteria:

- Treatment exists but the disorder is so rare that doctors and scientists have not been able to determine if all the above statements are true.
- Disease specific treatment exists, but it is not clear that making a diagnosis before signs and symptoms appear is better that starting treatment once an individual starts to have signs and symptoms.
- Disorders that do not cause health problems in a high percentage of people found to have a mutation (incomplete or variable penetrance).
- Signs and symptoms of the disorder usually start in later childhood or older, but in some cases do start in infants.
- Early research suggests that a treatment will improve health and quality of life, but more research is needed to know if the treatment will work for everyone with the disorder.
- Treatment exists but not everyone with the disorder has shown improvement.
- There is concern that an individual being diagnosed before signs and symptoms or who may never develop symptoms will be harmed in some way by that diagnosis.
- Genes that cause hearing loss, or cardiac malformations. Screening for hearing loss and heart malformations are part of the RUSP but may or may not be caused by gene mutation and knowing the specific gene that causes the disorder does not change how to care for the individual.

Once Group A and B disorders were established, molecular geneticists experienced in clinical genomic data curation and interpretation reviewed the Group A and B disorders for technical aspects that may limit the ability of NGS to identify pathogenic and likely pathogenic variants. This led to some conditions being moved to an additional group, Group C. Group C disorders will not be included in screened disorders on the CLIA approved test but will be held in reserve in the hope of adding them back once these technical issues can be resolved.

Overall, preliminary results from this and other pilot studies are very encouraging. Expanded study protocols are being developed to assess not only efficacy but cost analyses. The addition of the BeginNGS web-based tool will be essential to fill the knowledge gaps that

will undoubtedly be identified and can be used to guide initial interventions, confirmatory testing and longer-term management, particularly for those treatable diseases for which no specific/sensitive analyte-based newborn screening is currently available. As with GTRx, this web-based tool will be frequently updated with new treatments, and new conditions with the associated interventions.

Conclusion

The age of rapid whole genome newborn sequencing is upon us. It is imperative that this process be developed in a thoughtful, scientific manner that maximizes benefit while limiting harm. While current studies are evaluating efficacy of the test, it is important to remember that newborn screening is a program that can use this test, the purpose of which is to identify infants who will benefit from early intervention to improve outcomes. At the current time, national implementation in the United States is simply beyond the scope of the state health laboratories. This may be less true for nations with more uniform national newborn screening programs. Ultimately, it will need to be demonstrated that whole genome newborn screening is cost effective, saves lives and is acceptable to the general public, not just the medical/scientific community.

Conflict of Interest Statement:

S.F.K.is an employee and shareholder of Luna PBC, Inc. S.F.K. has filed a patent related to this work. The remaining authors have no competing interests.

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