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Review

Secondary Findings of Newborn Screening

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Abstract

The aim of newborn screening (NBS) program is to detect and manage treatable conditions in the early stages prior to the occurrence of long-term and irreversible sequalae. Phenylketonuria was the first screened disorder, but panels rapidly expanded after the introduction of tandem mass spectrometry technology into the program. Significant differences in the diseases screened by NBS were noted between programs in United States. Therefore, the recommended uniform screening panel was developed in 2006 to include a list of core disorders of NBS panels based on specific scoring system. Screening for these disorders may lead to incidental detection of secondary conditions. Identification of these conditions could be challenging due to unavailability of confirmatory testing, effective therapies and/or unclear natural history. In this review, we discuss several secondary findings of NBS and their associated disorders as well as the potential risk and benefits of their early diagnosis.

Keywords

Newborn screening; secondary conditions; incidental findings

1. Introduction

Newborn screening (NBS) is considered one of the most successful public health programs aiming to identify affected newborns with hereditary disorders in pre-symptomatic stage. The development of bacterial inhibition assay on dried blood spots by Robert Guthrie in the early 1960s enabled the first newborn screening for phenylketonuria [1]. Other metabolic disorders were included in the following years using the same assay [2-4]. In the 1990s, the use of tandem mass spectrometry (MS/MS) technology allowed the detection of several analytes simultaneously and therefore led to the expansion of NBS panel at low cost per sample, which is estimated at $10-20 per sample and <$1 for each additional test [5]. However, the NBS is a complex program consisting of multiple critical steps beyond the laboratory phase. Identifying positive results on NBS is followed by confirmatory testing, education and counseling and starting targeted therapies when available [6]. Guidelines and criteria were developed to justify the screening for certain conditions [7].

In response to the significant disparities in newborn screening services between states, the American College of Medical Genetics (ACMG) was commissioned by the Health Resources and Services Administration (HRSA) to develop guidelines for newborn screen programs in United States [8]. Scoring system with a cutoff value of 1200 separating high and low scoring conditions was developed based on survey responses and expert revision. As a result, a recommended uniform screening panel (RUSP) was published in 2006. The initial panel list involved 29 primary disorders based on certain criteria including the availability of early testing and effective management and known natural history. Detection of these primary conditions could lead to coincidental findings of 25 secondary conditions [8]. Reporting these findings was recommended if the associated conditions are known to be clinically significant, and diagnostic confirmatory tests are available to identify the underlying diagnosis [8].

With advancement of screening technology and development of new therapies, many conditions with initial low score had been moved to the core panel such as lysosomal storage disorders including Pompe disease and Mucopolysaccharidosis types I and II. As of January 2023, the list expanded to include 37 core disorders and 26 secondary disorders (Table 1), and the majority of states screen for these conditions [9]. However, some disorders can be incidentally identified through NBS, but they are not included in RUSP secondary conditions list yet. In this review, we describe some of these conditions and review the potential benefits and risks of expanding secondary conditions screenable by NBS programs. A list of these disorders including suggested 2nd tier testing are summarized in Table 2.

Table 1 The Recommended Screening Panel list of core and secondary conditions as of January 2023 [9].

	Core Conditions	Secondary Conditions
Organic Acid Disorders	Propionic acidemia	Methylmalonic acidemia with homocystinuria (Other cobalamin deficiency)
	Methylmalonic acidemia	Malonic acidemia
	Cobalamin disorders (Cobalamin A and B deficiency)	Isobutyrylglycinuria
	Isovaleric acidemia	2-Methylbutyrylglycinuria
	3-Methylcrotonyl-CoA carboxylase deficiency	3-Methylglutaconic aciduria
	3-Hydroxy-3-methyglutaric aciduria	2-Methyl-3-hydroxybutyric aciduria
	Holocarboxylase synthase deficiency	
	ß-Ketothiolase deficiency	
	Glutaric acidemia type I	
Fatty Acid Oxidation Disorders	Carnitine uptake defect	Short-chain acyl-CoA dehydrogenase deficiency
	Medium-chain acyl-CoA dehydrogenase deficiency	Medium/short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency
	Very long-chain acyl-CoA dehydrogenase deficiency	Glutaric acidemia type II
	Long-chain L-3 hydroxyacyl-CoA dehydrogenase deficiency	Medium-chain ketoacyl-CoA thiolase deficiency
	Trifunctional protein deficiency	2,4 Dienoyl-CoA reductase deficiency
		Carnitine palmitoyltransferase type I deficiency
		Carnitine palmitoyltransferase type II deficiency
		Carnitine acylcarnitine translocase deficiency

Amino Acid Disorders	Argininosuccinic aciduria Citrullinemia type I Maple syrup urine disease Homocystinuria Classic phenylketonuria Tyrosinemia type I Guanidinoacetate Methyltransferase Deficiency	Argininemia Citrullinemia type II Hypermethioninemia Benign hyperphenylalaninemia Biopterin defect in cofactor biosynthesis Biopterin defect in cofactor regeneration Tyrosinemia type II Tyrosinemia type III
Endocrine Disorders	Primary congenital hypothyroidism Congenital adrenal hyperplasia	
Hemoglobin Disorders	Sickle cell anemia βeta-thalassemia S,C disease	Other hemoglobinopathies
Others	Biotinidase deficiency Critical congenital heart disease Cystic fibrosis Classic galactosemia Pompe disease Hearing loss Severe combined Immunodeficiencies Mucopolysaccharidosis Type I Mucopolysaccharidosis Type II X-linked Adrenoleukodystrophy Spinal Muscular Atrophy due to homozygous deletion of exon 7 in SMN1	Galactoepimerase deficiency Galactokinase deficiency T-cell related lymphocyte deficiencies

Table 2 A list of secondary conditions discussed in this article.

Marker	Core Conditions	Secondary Conditions	Management Of Secondary Conditions	Suggested 2nd Tier Testing
Succinylacetone	Tyrosinemia type I	*FAH* pseudodeficiency or maleylacetoacetate isomerase deficiency	No treatment required	Molecular testing
Citrulline *(Low Level)*	Proximal urea cycle defects	Mitochondrial ATP synthase deficiency (MT-ATP6)	Citrulline and mitochondrial co-factors supplements improved neurological outcome in some reports when started early	Acylcarnitine profile: elevated C5-OH
C5-Hydroxyacylcarnitine (C5-OH)	3-hydroxy-3-methylglutaryl CoA lyase deficiency 2-methyl-3-hydroxybutyric acidemia β-ketothiolase deficiency 3-methylcrotonyl-CoA carboxylase deficiency 3-methylglutaconic aciduria Biotinidase deficiency Holocarboxylase synthetase deficiency	Mitochondrial ATP synthase deficiency (MT-ATP6)	Citrulline and mitochondrial co-factors supplements improved neurological outcome in some reports when started early	Plasma amino acids analysis: reduced citrulline level

Very Long Chain Fatty Acids	X-Linked adrenoleukodystrophy	Peroxisomal fatty acid oxidation defects such as Zellweger spectrum disorder, acyl-CoA oxidase deficiency and D-bifunctional protein deficiency	Supportive care	Plasma phytanic acid, plasmalogen and pipecolic acid
Lysosomal Enzymes Using Multi-Plex Enzyme Assay *(Low Levels)*	Mucopolysaccharidosis II, IIIA, IIID, IVA and VI	Multiple sulfatase deficiency	Supportive care	Molecular testing
Lysosomal Enzymes Using Multi-Plex Enzyme Assay *(High Levels)*	Mucopolysaccharidosis II, IIIA, IIID, IVA and VI	Mucolipidosis types II and III	Supportive care	Leukocytes or fibroblasts enzymes and plasma or serum LSD enzymes measurement and/or molecular testing
Lysosomal Enzymes Using Multi-Plex Enzyme Assay (High Levels)		Galactosemia Fructose intolerance Congenital disorders of glycosylation (CDGs)	Galactosemia and Fructose intolerance: Dietary management Congenital disorders of glycosylation (CDGs): supportive care; target therapies are available for some types	Galactosemia: GALT enzyme activity and/or galactose level Fructose intolerance: molecular testing CDGs: Molecular testing or enzyme activity for certain disorder. Plasma or serum carbohydrate deficient transferrin analysis (CDT) and plasma N-glycan analysis

T-Cell Receptor Excision Circles	Severe combined immunodeficiency (SCID)	22q11.2 deletion, syndrome, trisomy 21, ataxia telangiectasia, CHARGE syndrome, Noonan syndrome, Fryns syndrome, CLOVES syndrome, Nijmegen syndrome, EXTL3 deficiency, RAC2 deficiency	Supportive care	Chromosomal analysis and/or molecular testing
Beutler Method	Galactosemia	PGM1-CDG and glucose-6-phosphate dehydrogenase (G6PD) deficiency	PGM1-CDG: galactose supplement G6PD deficiency: avoidance of foods and medications that induce oxidative stress	PGM1-CDG: enzyme activity and/or molecular testing. Plasma or serum CDT and N-glycan tests G6PD deficiency: erythrocyte enzyme activity

2. Materials and Methods

2.1 Peroxisomal Disorders

X-Linked adrenoleukodystrophy is the only peroxisomal disorder included in the RUSP. It is due to hemizygous pathogenic variant in the ABCD1 gene located on X chromosome that impairs the peroxisomal transmembrane transport protein for very long chain fatty acids (VLCFAs). Consequently, VLCFAs accumulate in the plasma and tissues [10, 11]. Affected males may present with variable phenotype including primary adrenal insufficiency in early childhood, adulthood adrenomyeloneuropathy and childhood cerebral form which is considered the most severe and fatal phenotype if left untreated [12]. Hematopoietic stem cell transplant has been effective in halting the progression of disease when it is performed in early stages [13, 14]. Therefore, identifying the disease early is essential for successful treatment. New York was the first state to include X-ALD in their NBS program in 2013 by adopting a three-tiered algorithm, starting with detection of C26:0 lysophosphatidylcholine (C26:0) via high-throughput flow injection analysis tandem mass spectrometry (FIA-MS/MS). Samples with out-of-range results are rescreened using LC-MS/MS [15]. The third tier testing consists of ABCD1 gene sequencing [16]. New York was followed by other states such as Connecticut and California in including X-ALD in their panels. In 2015, the nomination to add X-ALD to the RUSP was approved [17]. However, identifying X-ALD through NBS is associated with several potential challenges when counseling the families of newborns with positive results. There is no established gene-phenotype correlation, and the same genotype could be associated with variable clinical course including intra-familial variability [16]. Detecting the disorder in a female newborn is another challenge as adrenal involvement is very rare in heterozygous females, but they are at risk of developing adrenomyeloneuropathy at age of 60 years [17].

Given that carrier females are not at early risk for developing a disease, Netherland NBS program added a 2nd tier testing to determine the number of X chromosome of screened newborn when C26:0 is elevated on first testing. If only one X chromosome is detected, then screening proceeds to 3rd and 4th tier consisting of HPLC-MS/MS for C26:0 and ABCD1 gene sequencing, respectively [18].

Other disorders of peroxisomal fatty acid oxidation such as Zellweger spectrum disorder (ZSD), acyl-CoA oxidase deficiency and D-bifunctional protein deficiency are associated with elevated C26:0 and can be identified incidentally when screening for XALD [19]. In addition, C26:0 elevation can also be seen in some neonates with Aicardi-Goutieres syndrome [20]. ZSD is associated with the highest incidence among these secondary findings with an estimated frequency ranging from 1:12,000 in the French Canadian region of Quebec, 1:50,000 in North America, to 1:500,000 in Japan [21-23]. It is considered as a continuum of heterogenous phenotypes with multisystemic involvement secondary to defects in one of the PEX genes [24]. Core features include liver disease, neurological abnormalities, and vision and hearing impairment [21]. Hearing loss is a common finding in ZSDs and affected infants may fail their newborn hearing screening test [25]. Newborns with elevated C26:0 and negative ABCD1 gene sequencing on NBS should be referred to specialists to rule out other peroxisomal disorders [19]. If the infant also failed the hearing screen, the suspicion of ZSDs should be high. Follow-up testing includes plasma or serum very long chain fatty acids (VLCFA) and branched -chain fatty acids (BFAs), red blood cell plasmalogen, and plasma or urine pipecolic acid. Severe ZSDs have increased C26:0, phytanic acid, reduced plasmalogen and increased pipecolic acid. Some severe ZSDs can also have hyper-oxalic aciduria detectable by urine

organic acid test [21]. In plasma acylcarnitine profile, severe ZSD can have increased dicarboxylic C16-, C18-carnitine as well as C26:0-carnitine [26]. Although, there is no targeted therapy currently available for these conditions and their inclusion in NBS panels does not meet the screening criteria, majority of these are pediatric diseases that often presents in early infancy [21]. Thus early diagnosis from newborn screening follow-up facilitates the disease management and allow early opportunity for family counseling [17]. With the advancement of gene therapies, early diagnosis also provides opportunities for developing future treatment.

2.2 Mitochondrial Disorders

Pathogenic variants in MT-ATP6 was the first described Complex V defect resulting in mitochondrial ATP synthase deficiency [27]. ATP6 encodes one of the two functional domains of ATP synthase (or Complex V) that is involved in mitochondrial oxidative phosphorylation converting ADP and inorganic phosphate to ATP [28]. MT-ATP6 disease is associated with variable clinical features ranging from neuropathy, ataxia and retinitis pigmentosa (NRP) syndrome to early onset leigh syndrome. The severity and age of onset correlate with the level of heteroplasmy. However, carriers with low heteroplasmy level may have subtle symptoms [29]. The m.8993T>G is the first reported pathogenic variant and associated with a decreased ATP synthesis [27], but pathogenic mechanism varies between mutations [29]. Since the first description of m.8993T>G variant in 1992, more than 500 cases of ATP6-associated disease have been reported, among these more than 100 cases carry m.8993T>C mutation [30]. The affected individuals usually are asymptomatic at birth but several reports have linked abnormal NBS results of low citrulline and/or elevated C5-OH with this disorder. Some patients had an elevated C3 acylcarnitine on their NBS or follow up testing [31, 32].

Both citrulline and C5-hydroxyacylcarnitine (C5-OH) are listed as markers in the primary RUSP. High citrulline is associated with distal urea cycle defects including Argininosuccinic aciduria and Citrullinemia type I as well as Citrullinemia II and Pyruvate carboxylase deficiency [6]. Low citrulline, on the other hand, is reported by only few NBS programs due to its low positive predictive value for proximal urea cycle defects [33].

The selected transition monitoring used for C5-OH represents 3-hydroxyisovalerylcarnitine or 2-methyl-3-hydroxybutyrylcarnitine [34]. Elevated C5-OH in NBS is used as a marker for some inborn errors of metabolism of branched chain amino acids or ketones such as 3-hydroxy-3-methylglutaryl (HMG)-CoA lyase deficiency, 2-methyl-3-hydroxybutyric acidemia (2M3HBA), β-ketothiolase deficiency, 3-methylcrotonyl-CoA carboxylase (3MCC) deficiency and 3-methylglutaconic aciduria (3MGA) and biotin defects including biotinidase deficiency and holocarboxylase synthetase deficiency [6].

The exact mechanism of these metabolites abnormalities in affected individuals with MT-ATP6 is not well established. However, reduced citrulline level could be due to dysfunction of the ATP-dependent enzymes, CPS1 and pyrrolone-5-carboxylate synthetase, involved in proximal urea cycle and therefore, affecting the intestinal citrulline synthesis [31, 35, 36]. The abnormal acylcarnitine profile could be secondary to carbonic anhydrase (CA5A) dysfunction in ATP6 deficiency [31].

On confirmatory studies of identified newborns, urine organic acids showed elevated lactate, 3-hydroxyisovalerate, 3-hydroxypropionate, 3-methylcrotonylglycine, methylcitrate, propionylglycine,

2-methyl-3-hydroxybutyrate in some individuals [29, 37]. But the initial urine organic acids analysis in the newborn period might be normal [37].

Identification of these patients in the newborn period could be beneficial not only to establish the underlying diagnosis but also to start early treatment before neurological sequalae. Citrulline and mitochondrial co-factors supplementation improved the neurological outcome in a cohort identified via NBS [32]. Thus increased C5OH/citrulline or C3/citrulline ratio or increases in both ratios in NBS could trigger a positive screen for MT-ATP6 m.8993T>G or CA5A deficiency. In addition, plasma amino acid tests should be included in the follow-up test algorithm of NBS positive for C3 and/or C5OH. Conversely, plasma acylcarnitine test should be included in the follow-up testing algorithm of NBS positive (in certain states) for low citrulline or high glutamine/citrulline ratio. For CA5A deficiency, early diagnosis and intervention can potentially prevent hyperammonemia and save lives [38].

2.3 Lysosomal Storage Disorders

Lysosomal storage disorders (LSD) are group of progressive disorders secondary to deficiency of one of hydrolases enzymes located within the lysosomes. Management including enzyme replacement, substrate reduction and hematopoietic stem cell transplant is available for some of these conditions [39, 40]. However, early detection is essential to prevent or at least slow the progression of irreversible organ damage [40]. Using a multi-plex enzyme assay via LC-MS/MS enables the inclusion of multiple LSDs in NBS programs and therefore, facilitates their diagnosis in pre-symptomatic stage [41, 42]. Storage and shipping environment such as heat and humidity may affect the enzyme activity assay and results in falsely reduced level [43], particularly if stored for prolonged time [44]. Among LSD, MPS types I and II were added to the RUSP in 2016 and 2022, respectively [45, 46]. However, secondary disorders could be identified through this multiplex assay screening method. One of these disorders is multiple sulfatase deficiency which is an autosomal recessive disease due to pathogenic variants in SUMF1 gene that encodes formylglycine-generating enzyme [47]. This enzyme is responsible for post-translational activation of sulfatases in endoplasmic reticulum; and when this enzyme is deficient, dysfunction of all sulfatases occurs including the enzymes associated with MPS II, IIIA, IIID, IVA and VI [47-49]. The incidental detection of this disorder could lead to early diagnosis, although currently there is no targeted therapy and the management is mainly supportive [50]. However, gene therapy for multiple sulfatase deficiency is also underway.

Mucolipidosis types II and III (ML II/III) are caused by defective uridine-diphosphate N-acetylglucosamine: lysosomal-enzyme-N-acetylglucosamine-1-phosphotransferase which is essential for addition of the targeting signal (mannose-6-phosphate) to the glyans in lysosomal enzymes [51, 52]. This defect leads to elevated plasma and whole blood levels of mannose-6-phosphate (M6P) dependent lysosomal enzymes which can be detected via NBS tests for other LSDs [42]. These enzymes activities are normal or reduced when measured in leukocytes and fibroblasts by NBS follow-up tests [53].

Other conditions, including treatable ones, could have similar biochemical profile to ML II/III and elevate the lysosomal enzymes levels on plasma with no effect on their levels in leukocytes and fibroblasts [54]. These disorders include fructose intolerance, congenital disorders of glycosylation and galactosemia [54, 55]. However, enzymes levels usually normalize after initiation of dietary

therapy [55]. These disorders affect the glycosylation and the cause of the increased serum enzymes levels is complex; and it is thought to be related to defective M6P targeting of enzymes into lysosomes or reduced their stability and cellular uptake [56].

3. Discussion

The main purpose of the NBS is detecting treatable hereditary disorders in the pre-symptomatic stage to halt the progression of disease and prevent irreversible damage. However, expanding the NBS panels at relatively low cost led to the discovery of other conditions that lack targeted therapy or has no known significant clinical impact. Tyrosine, for example, has been used as a marker for tyrosinemia type I secondary to pathogenic variant in FAH gene. But affected newborns with this disorder could have normal tyrosine level and normal NBS results if only tyrosine is used as a marker for this disorder [57]. Elevated tyrosine is also not specific and associated with other conditions such as prematurity, hepatic dysfunction, or total parental nutrition [58, 59]. In contrast, succinylacetone (SA) is very specific metabolite for tyrosinemia type I and has been included in many NBS programs [57, 60]. But recent reports have identified mild elevation of SA in newborns not affected by tyrosinemia type 1 and they were found to have FAH pseudodeficiency or maleylacetoacetate isomerase deficiency [61, 62]. The latter is due to pathogenic variants in GSTZ1 gene and thought to be a biochemical abnormality with no clinical manifestations. Six affected individuals identified through NBS were followed for up to 13 years and continued to show no hepatic or renal dysfunction while being on unrestricted diet [62]. Detecting SA in such conditions may cause parental anxiety and necessitates molecular testing and prolonged follow-up to rule out tyrosinemia type I. However, missing tyrosinemia type I diagnosis via NBS by using non-specific marker increases the risk of poor outcome with irreversible damage [63].

In contrast, other assays are known to be associated with high false positive rate such as using T-cell receptor excision circles (TRECs) used for severe combined immunodeficiency (SCID) screening [64]. TRECs are small circular DNA formed during T-cell rearrangement and can be detected on dried blood spot [65]. Wisconsin was the first state piloted the neonatal screening for SCID in 2008 [66], and it is currently included in the RUSP core conditions list given that early management by hematopoietic stem cell transplant, ERT or gene therapy for certain types are available and improve the outcome [67, 68]. However, other conditions associated with lymphopenia have been identified through abnormal TREC assay such as 22q11.2 deletion syndrome, trisomy 21, ataxia telangiectasia, CHARGE syndrome among others [69]. Prematurity is another cause of false positive results [70]. If SCID workup is negative, these infants with abnormal TERCs results on NBS should be evaluated by a specialist to rule out other causes of lymphopenia. Thus if the secondary finding is a known pediatric disease with early infantile presentation, both short-term and long-term clinical benefits can easily outweigh the cost [68-70].

Using certain assay to screen for a group of disorders could increase the risk of detecting secondary findings. For example, Beutler method has been developed for screening of galactosemia. It measures the fluorescence signal of NADPH to determine the activity of GALT enzyme that is deficient in galactosemia. However, the NADPH is produced during the metabolic process of Glucose -1-phospahate, a GALT product, by the stepwise activities of three enzymes: phosphoglucomutase-1 (PGM1), glucose-6-phosphate dehydrogenase (G6PD), and 6-phosphogluconate dehydrogenase (6PGD) [71]. Deficient activity of any of these enzymes could lead to decreased NADPH signal and

results are interpreted as a false positive galactosemia case [72, 73]. Therefore, a minor assay optimization and validation can be used to screen G6PD or PGM1-CDG by Beutler method with little additional cost to NBS. Conversely, severe form of PGM1 and G6PD deficiency may be picked up incidentally by the current Beutler assay. Thus it is important to confirm galactosemia through enzymatic activity and/or molecular studies in cases of abnormal NBS using Beutler assay in a timely fashion. Importantly, galactosemia management by restricting galactose intake and using soy formula could be harmful in individuals affected by PGM1-CDG or G6PD deficiency [72, 74] which emphasize the importance of testing for secondary conditions.

The recent advancement of molecular technology allowed the development of several ongoing genome sequencing newborn screening programs worldwide such as Screen4Care in Europe, Baby Detect in Belgium and BabyScreen+ in Australia. These programs are using either genome sequencing for target panel of genes or exome sequencing [75]. In US, the babySeq project is a series of prospective NIH-funded clinical trials; a total of 159 newborns (127 healthy newborns and 32 sick newborns) were randomized to receive genome sequencing in the first phase of the study. The reported findings included affected or carrier status of pathogenic and likely pathogenic variants in genes associated with highly penetrant childhood disorders or moderately penetrant disorders with evidence of improved outcome associated with early intervention. Pharmacogenomic variants for medications relevant to use in pediatric population were also added [76]. 15/159 newborns were identified to be at risk of childhood disorder. Three of these newborns were affected by disorders related to NBS but missed by conventional screening methods, including KCNQ4-related postlingual hearing loss not detectable by hearing screening at birth, partial biotindase deficiency and non-classical congenital adrenal hyperplasia due to compound heterozygous variants in CYP21A2 known to be associated with delayed onset manifestations. Carrier status was identified in 140 newborns (88%) and pharmacogenomics variants in DPYD, TPMT and G6PD were detected in 8 newborns (5%) [77]. However, there are several ethical and technical issues concerning the genome sequence screening including parental testing to identify phasing for detected compound heterozygous variants, reporting conditions associated with low penetrance, variable or adult onset phenotype or detecting an X-linked disorder in a female infant [78]. The longer turnaround time and lower sensitivity and specificity compared to biochemical screening method should also be considered [79]. Though the genomic sequence screening are not thought to replace the conventional NBS, molecular testing could confirm the disorders associated with secondary findings in NBS avoiding the diagnostic delay and decreasing parental anxiety. As more NBS conditions require molecular genetic confirmation, it is a matter of time that high through and high quality genome sequencing technology will be used in NBS program. We foresee that the implementation of whole genome sequencing will further facilitate the discovery and expansion of secondary NBS conditions.

4. Conclusions

Expanding NBS has been possible at low cost with the recent advances in the technology. However, other factors should be carefully considered when adding a specific disorder. Establishing early diagnosis for untreatable and late onset pediatric conditions could avoid diagnostic odyssey but it causes undesired anxiety. Easy access to specialist for evaluation and confirmatory testing followed by counseling are essential to address family concerns and guide them in their decisions. Gene therapy is rapidly evolving and provides a promising treatment modality for many metabolic

disorders that are considered untreatable. Success in this filed will help justifying adding other disorders to the NBS in the future. Conversely, early diagnosis of secondary conditions can also provide opportunities and support for the development of clinical trial readiness.

Author Contributions

HA wrote the manuscript. MH revised and edited the manuscript. Authors discussed the content and approved the submitted version.

Competing Interests

The authors have declared that no competing interests exist.

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