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Review

Newborn Screening for Genetic Diseases: An Overview of Current and Future Applications

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Abstract

Newborn screening (NBS) for inborn errors of metabolism (IEM) was introduced more than 50 years ago with the testing of phenylketonuria (PKU) using blood spots deposited on a filter paper after heel prick. NBS aims to identify early after birth inherited disorders for which clinical management and pre-symptomatic treatment will significantly decrease morbidity and mortality. While NBS for a few other disorders was implemented in some specific jurisdictions over the following decades, it is with the introduction of tandem mass spectrometry (MSMS) by the end of the 1990's that NBS expansion really took place. MSMS has had a tremendous impact on NBS, but led to heterogeneity in NBS disorders panel between jurisdictions that have introduced new conditions at various rates using different decision-making processes. In addition to disorders tested by MSMS, many programs have included other inherited disorders using various testing methodologies, including cystic

fibrosis (CF), sickle cell diseases (SCD), while some others have been recently introduced in some jurisdictions or are under considerations, such as severe combined immunodeficiency (SCID), lysosomal storage disorders and X-linked adrenoleukodystrophy (X-ALD). As the cost of DNA testing decreases, the future of NBS will inevitably include genome sequencing, which will represent an opportunity to detect, treat and prevent more serious early-onset health conditions in the best interest of newborns, provided that public health authorities rule on ethical and policy issues, such as the identification of variant of unknown significance and the disclosure of incidental findings. Finally, despite the current exciting technological advances in the field of NBS in the Northern hemisphere, we should not be blinded that despite various initiatives, about two-thirds of neonates born in low-resource areas are still waiting to have access to NBS**.**

Keywords
Newborn screening; genetic diseases; next-generation sequencing; low-resource areas

1. Newborn Screening Then and Now: An Overview

In 1961, Dr Robert Guthrie described a method of testing newborns for Phenylketonuria (PKU). The first disorder screened for, PKU, is a rare, recessively inherited enzyme deficiency of the phenylalanine hydroxylase that generally results in severe mental retardation unless treated from early infancy [1]. Dr Guthrie proposed a simple blood sampling method that involved phenylalanine measurement from heelstick blood samples dried on a filter paper. The advantages of dried blood spots on filter paper are that sample collection is easier and that logistic aspects related to sample storage and shipment through postal services to a central laboratory for testing can be relatively limited, without the need of a refrigerator or dry ice [2].

Rapidly, US states collaborated to the implementation of universal screening, and already in 1963 over 400 000 newborns from 29 states were screened, which revealed 37 cases of PKU. All affected children were on low phenylalanine dietary treatment by the age of 1 month, and the feasibility of mass screening was clearly shown [3]. By 1965, 32 states performed newborn screening (NBS) in the USA, including 27 states enforcing mandatory screening [4]. In the following years, many other countries from the western world implemented newborn screening for PKU. The next breakthrough in newborn screening came in 1975 when Jean Dussault published a method of newborn screening for congenital hypothyroidism (CH) using the same filter paper in use for PKU [5]. Most developed countries had adopted screening for PKU and CH in the 1970's.

In the next two decades, there were a very few initiatives to add other disorders to NBS programs, such as congenital adrenal hyperplasia (CAH), haemoglobinopathies, biotinidase deficiency and cystic fibrosis (CF) [6]. Some of the additional disorders added to the limited NBS panel included those for which there was a high prevalence in the related jurisdiction. As an example, NBS for hereditary tyrosinemia type 1 (HT1) due to fumarylacetoacetate hydroxylase deficiency, was added in 1970 in Québec where a genetic founder effect in the French-Canadian population led to an increased prevalence of the disorder [7]. HT1 was first screened by tyrosine

measurement, but eventually succinylacetone, a byproduct, which showed to be more sensitive and specific and became the 1st tier screening test for HT1.

In the 1990s the development of electro-spray ionisation tandem mass spectrometry (MSMS), a multiplex platform technology allowing multianalyte screening from one blood spot, and thus the simultaneous detection of many rare inherited disorder after only one single injection into the instrument, led to the massive expansion of NBS [8]. Since then, many jurisdictions have progressively performed their own decision-making process for the expansion of NBS, which led to heterogeneous NBS disorder panels. Some countries adopted a very conservative approach including only a few disorders, while others have increased significantly the number of conditions screened to more than 30 in their NBS panel. To add to the complexity, some countries such as the United States and Canada do not have nationally mandated NBS programs, as they fall under territorial (states, provinces,…) jurisdictions, each of which may follow their own processes of NBS expansion. In the United States, however, to decrease state-to-state heterogeneity between NBS disorders panels, which raised equity concerns of advocacy groups [9], the Secretary of Health and Human Services' Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC) mandated the American College of Medical Genetics to provide the Secretary with national NBS recommendations for a Recommended Uniform Screening Panel (RUSP): out of 88 conditions assessed, this led to the adoption of a core of 29 conditions to be screened and 25 secondary target conditions [10]. The adoption of the RUSP in the United States led to significant harmonization, although the methodology used was debated [11, 12]. Since the initial version, other conditions, such as severe combined immunodeficiency disease (SCID) [13] [14], X-linked adrenoleukodystrophy, critical congenital heart disease (CCHD)[15](performed by pulse oximetry), Pompe disease, and Mucopolysaccharidosis type I (MPS I), as well as spinal muscular atrophy due to homozygous deletion of exon 7 in SMN1 and newborn hearing screening [16] have been included in the core conditions of the RUSP, which now represents 35 conditions [17].

Of note, screening techniques for lysosomal diseases are also rapidly developing. A tandem mass spectrometry assay in which the enzymatic activities of 9 lysosomal enzymes (α-glucosidase, α-galactosidase A, α-l-iduronidase, acid sphingomyelinase, acid β-glucocerebrosidase, galactocerebrosidase, iduronate-2-sulfatase, N-acetyl-galactosamine-6-sulfatase, N-acetyl-galactosamine-4-sulfatase) was developed to screen Pompe disease, Fabry disease, Hurler disease, Niemann-Pick-A/B disease, Gaucher disease, Krabbe disease, mucopolysaccharidosis II, IVA and VI, respectively [18]. Peroxisomal diseases as X-linked adrenoleukodystrophy are also studied using LC-MSMS [19]. Some conditions, such as Duchenne muscular dystrophy are not included in the core conditions of the RUSP but are screened in newborns in some jurisdictions [20]. Approved treatments for Duchenne muscular dystrophy may change the natural history of this disease [21]. To avoid diagnostic delay and provide optimal treatments, early identification of patients through NBS will be necessary.

If the ACMG recommendations had some influence in the decision-making process outside the United States, they did not receive worldwide support, most probably because different jurisdictions evaluate relevance of conditions to be introduced in their NBS programs using different angles, which leads to various interpretations of similar criteria. In their 1968 World Health Organization report, Wilson and Jungner identified 10 principles and practice of population screening to guide the selection of conditions that would be suitable for screening (Table 1) [22]. Population screening aims to identify disorders that fulfill those criteria that are mainly based on

the severity and natural history of the disorder, the capacity to detect the condition at an early pre-symptomatic stage using an acceptable test, and the availability of an acceptable treatment. Also, they considered the impact of screening on the cost of case-finding, which should be economically balanced in relation to medical costs. Considering the many technological advances that were introduced since, which made screening a topic of growing importance and controversy, an updated WHO report was published forty years after the classic Wilson and Jungner criteria were outlined [23]. The WHO criteria were not specifically directed for genetic application and newborn screening, but they have stood the test of time. While there is a broad consensus on these population-based screening criteria, there is variability in how they are interpreted. As mentioned by Wilcken and Wiley regarding NBS, there is in fact little hard evidence about the benefits of screening [6], which probably explains, as least for a significant part, the differences in the evaluation of the relevance of conditions to be screened between the different territories [24]. One must not only consider the benefits, but also the potential harms for each condition added to a NBS panel, namely the increase of false positive results leading to unneeded interventions, as well as the potential parental psychological distress and potential distortion of parental perception about the child, while expanded screening may give rise to so called "patient in waiting", i.e. individuals with a genetic diagnosis who remain asymptomatic for years or decades [25]. After performing a systematic review of the literature on NBS criteria worldwide, Jansen *et al.* concluded that despite commonalities, NBS programs are using different approaches to explore the same policy issues, including: the beneficiary of NBS, definition of criteria, the way conditions are assessed, level of evidence required, and recommendations after assessment, which potentially results in increased disparity across NBS programs internationally [26].

Table 1 Summary of the 1968 Wilson and Jungner criteria for screening, [22] (updated by Andermann *et al.* in 2008 [23]).

Principal Wilson and Jungner Criteria for Screening
• **Condition is an important health problem**
• **Acceptable treatment available**
• **Facilities for diagnosis and treatment available**
• **Knowledge of the natural history of the disorder**
• **Presymptomatic intervention improves outcomes**
• **Simple and low cost test with excellent performance**
• **Resources available for follow-up of positive individuals**
• **Program acceptable to the population**
• **Development of a program policy**
• **Cost of case-finding is acceptable**

2. Next Generation Sequencing in the Field of NBS: Potential and Concerns

With the recent sequencing of the entire human genome [27, 28], genetic screening is being proposed as a major opportunity for translating genetic and genomic advances into population health gains [29, 30]. Next generation sequencing (NGS) has already started to transform genetics, and clinical application of whole sequencing genetics has already shown to decrease the diagnostic odyssey in both adults and children [31, 32] and shortening intensive care units stay in critically ill children [33]. With decreasing costs and novel genotyping and sequencing technological platforms, interest in the use of molecular testing for NBS is increasing. However, DNA testing has already been introduced in NBS for several years for some disorders. As an example, most programs performing NBS for cystic fibrosis have implemented a *CFTR* mutation panel as a second-tier test following increased immunoreactive trypsin level. Considering the capacity to perform DNA testing from filter paper combined with the decreasing costs of DNA testing, there is growing interest for including more widely DNA testing technologies to NBS, which would greatly increase the capacity to identify rare but devastating disorders, but which also raises some ethical concerns.

Again, gene testing is used as a 2nd tier test following a positive biochemical screen (i.e. cystic fibrosis in most NBS programs offering NBS for CF) and as part of the diagnostic confirmation workup of many conditions. However, there is yet no genetic conditions screened using 1st tier gene sequencing. Theoretically, 1st tier targeted NGS would allow the systematic sequencing of a panel of genes for detecting all the conditions of a NBS panel to be detected at birth. As an example, without taking into account 3 pathologies, newborn hearing screening, critical congenital heart defect and congenital hypothyroidism (for which there is great genetic heterogeneity)[34], this would represent a panel of 57 genes when applying the ACMG recommended “core panel” (Table 2). However, the more a condition is genetically complex, the more a biochemical approach would be favored, provided that the latter is highly sensitive and specific. Also, some biochemical tests and markers can detect more than one condition. For example, an increase in the phenylalanine level may be caused by classical phenylketonuria due to a defect in phenylalanine hydroxylase, but may also unravel a deficit in the metabolism of tetrahydrobiopterin [35]. Similarly, an elevation of the 17 hydroxy progesterone may be caused by a 21-hydroxylase deficiency but also other steroidogenesis enzymes.

Table 2 Main genes involved in core panel of diseases to be detected at birth according to ACMG newborn screening expert group (HGNC, HUGO Gene Nomenclature committee).

Group of conditions	Condition	HGNC approved gene symbol (OMIM)
Organic acidemias	Isovaleric acidemia	*IVD (607036)*
	Glutaric acidemia type I	*GCDH (608801)*
	3-OH 3-CH3 glutaric aciduria	*HMGCL (613898)*
	Holocarboxylase synthase deficiency	*HLCS (609018)*
	Methylmalonic acidemia (mutase deficency)	*MMUT (609058)*
	3-Methylcrotonyl-CoA carboxylase deficiency	*MCCC1 (609010), MCCC2 (609014)*
	Methylmalonic acidemia (cobalamin disorders)	*MMAA (607481), MMAB (607568), MMADHC (611935)*
	Propionic acidemia	*PCCA (232000), PCCB (232050)*
	β-Ketothiolase deficiency	*ACAT1 (607809)*
	Biotinidase deficiency	*BTD (609019)*
Disorders of fatty acid metabolism	Medium-chain acyl-CoA dehydrogenase deficiency	*ACADM (607008)*
	Very long-chain acyl-CoA dehydrogenase deficiency	*ACADVL (609575)*
	Long-chain L-3-OH acyl-CoA dehydrogenase deficiency	*HADHA (600890)*
	Trifunctional protein deficiency	*HADHB (143450)*
	Carnitine uptake defect	*SLC22A5 (603377)*
	Carnitine transport defect	*CPT1A (600528), SLC25A20 (613698), CPT2 (600650)*
Disorders of amino acid metabolism	Phenylketonuria	*PAH (612349)*
	Maple syrup (urine) disease	*BCKDHA (608348), BCKDHB (248611), DBT (248610), PPM1K (611065)*
	Homocystinuria (due to CBS deficiency)	*CBS (613381)*
	Citrullinemia	*ASS1 (603470)*

	Arginosuccinic acidemia	*ASL (608310)*
	Tyrosinemia type I	*FAH (613871)*
Hemoglobinopathies	Sickle cell anemia (Hb SS disease)	*HBB (141900)*
	Hb S/β-thalassemia	
	Hb S/C disease	
Endocrinopathies	Congenital hypothyroidism	*DUOX2 (606759), DUOXA2 (612772), PAX8 (167415), TG (188450), TPO (606765), TSHR (603372), IYD (612025)…*
	Congenital adrenal hyperplasia (21-hydroxylase deficiency)	*CYP21A2 (613815)*
Others metabolic conditions	Glycogen storage disease type II (Pompe)	*GAA (606800)*
	Mucopolysaccharidosis type 1	*IDUA (252800)*
	X-linked adrenoleukodystrophy	*ABCD1 (300371)*
	Classical galactosemia	*GALT (606999)*
Others	Spinal muscular atrophy due to homozygous deletion of exon 7 in SMN1	*SMN1 (600354)*
	Newborn hearing screening	*/*
	Cystic fibosis	*CFTR (602421)*
	Critical congenital heart disease	*/*
	Severe combined immunodeficiencies	*ADA (608958), CARD11 (607210), CD247 (186780), CD3D (186790), CD3E (186830), CORO1A (605000), CTPS1 (123860), DCLRE1C (605988), IKBKB (603258), IL2RG (308380), IL7R (146661), JAK3 (600173), LAT (602354), LCK (153390), PRKDC (600899), PTPRC (151460), RAG1 (179615), RAG2 (179616)*

In a recent report, Yang *et al.* performed systematic NGS in biochemically screened-positive neonates by MSMS from three areas of the Jiangsu Province performing NBS for 27 inborn errors of metabolism (IEM). Over a period of four years, 536,008 neonates had positive 1st tier tandem MS screen followed by MSMS retesting and targeted sequencing of 306 IEM-related genes from an

extended panel of inherited metabolic diseases. A total of 1033 neonates were tested by NGS, followed by validation analyses by Sanger sequencing of positive findings. They identified the disease-causing genes of 194 cases for an overall incidence of 1:2763, which was higher than previously reported and suggesting that NGS allowed to identify additional cases [36]. Aminoacidopathies accounted for 43.5% of case findings, while 34.8% were organic acidemias and 21.7% were fatty acid oxidation disorders. Most cases needed clinical intervention and regular follow up, while only a few cases appeared to be asymptomatic at the time of publication. Long term follow up should allow to adjust conditions and genes to be included as part of the gene panel in routine NBS.

The high throughput of NGS technology, which can carry out parallel sequencing of millions of DNA molecules, has the potential to increase the number of congenital disorders to be screened, in fact to a much higher order of magnitude seen with the introduction of tandem MS. In this context, methods for the selection of appropriate conditions to be introduced as part of a NGS NBS-panel must be developed and tested. With this in mind, Milko *et al.* recently developed an aged-based semi-quantitative scoring method for evaluating the relevance for the introduction of new genetic conditions to NBS panel using genomic sequencing [37]. Their model was mostly based on major screening criteria to evaluate clinical actionability (severity of the disease, likelihood of clinical manifestation, acceptability and efficacy of intervention, overall knowledge of the gene-disease association) [38]. This allowed them to identify 822 gene-disease pairs, with 466 classified as being children-onset with high clinical actionability, while another 245 gene-disease pairs were considered as low to no childhood actionability. Of note, validation of their method to reliably identify pediatric clinical actionability was performed by comparing how the 1^{ry} and 2^{ry} conditions of the RUSP [39] and conditions curated by the BabySeq project [40] performed with their approach. As expected, the correlations were not perfect, but they reflected the lower clinical actionability of 2^{ry} compared to 1^{ry} RUSP conditions, more of the latter being classified in the high clinical actionability category. Comparisons with the BabySeq projects, which categorized their gene-disease pairs on the validity of the gene-disease association, earliest reported age of onset, and penetrance, allowed to reach to a consensus for the identification of 292 conditions to be potentially introduced to a NGS NBS-panel, and another 125 optional for disclosure [37]. Considering that the benefit of NBS for many of the 466 disease-pairs is yet to be proven, it would be wise to apply a stringent classification restricting NGS NBS only for disorders showing definitive or strong clinical validity based on the ClinGen framework [41]. Of note, such genomic-based approaches may allow to help decision process about the debatable relevance of conditions such as 3-methylcrotonylglycinuria that are already part of the NBS panel of many jurisdictions.

The gap between what is technologically possible by NGS and the capacity to offer appropriate services by public health systems is creating pressure to expand screening programs, often before adequate regulatory frameworks are in place [42-44]. Indeed, concerns are mainly related to psychological and physical consequences of broad genetic testing in asymptomatic children, such as parental anxiety in the presence of indeterminate findings, loss of confidentiality, and social stigma [25, 45-48]. This is thus the subject of intense debates, especially in the absence of an appropriate medical intervention to offer. The generation of genomic information, not all of which is understood, potentially leads to significant risks associated with the release of incidental findings and variant of unknown significance (VUS). This new paradigm generated by the introduction of NGS in NBS must be taken into account by an appropriate public health

framework, as recommended by Friedman *et al*. [44]. While some are already proposing that NBS programs incorporate, or even replace their current approach with genomic sequencing [49, 50], evidence-based methods should be implemented to inform the decision-making process and consider parameters such as prevalence and penetrance of the condition, false-positive findings, clinical outcome following the interventions, psychological impacts on families, and costs. In the case of NBS, the focus should be the best interest of the child, and thus the identification of conditions where timely interventions or prevention would prevent significant health harms to the child, not on genetic information that may potentially identify later-onset disorders which might need medical attention. Otherwise there would be a denial of the autonomy of the individual. The introduction of NGS in NBS carries an immense potential to better identify children with genetic disorders and improve outcomes, but also poses important ethical issues. It is not surprising that it is the subject of active discussions and debates.

3. NBS in Low-Resource Areas: An Overview

Even if NBS is a considered as one of the major public health realization [51], unfortunately many countries in low resource-areas do not have a structured national program. While the incidence of congenital disorders is estimated at 8 million of annual birth worldwide, only one-third of neonates are receiving NBS for at least one inborn disorder [52]. In addition to the lack of funding and of specialized human resources, NBS advocates face many barriers to NBS implementations. Also, the centralized model of NBS based on the shipment of collection papers to a central NBS laboratory may not be suitable in these areas. In the absence of a structured public health system that can insure effective care and follow up of screen-positive neonates, NBS may not be viable.

Indeed, NBS includes a whole series of activities and a sequence of actions, it is not only a testing laboratory. Blood collection and transport to a central laboratory, follow up investigations and treatments of those with an abnormal result requires a structured public health system and adequate infrastructure. In addition, the different realities of outlying and urban areas make it difficult to implement a uniform NBS program. This was emphasized in India where it was suggested that NBS for congenital hypothyroidism only be introduced in the countryside, while screening should be implemented for congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH) and G6PD deficiency in urban areas, tandem mass spectroscopy being reserved only for investigating the high-risk cases. This must hold true for most of the developing countries [53].

In addition, health care authorities may not see NBS of rare conditions, sickle cell diseases aside for some territories, as a priority. This will possibly be attenuated as more neonatal and children's suffering from more common disorders are being taken care of. Apparently, screening for genetic disorders and birth defects becomes relevant when infant mortality rate falls below 40 per 1000 live births [54], and become a public health priority when national infant mortality rate drops into single digits, i.e. below 10 per 1000 live births [54, 55]. However, differences in the population genetic background will inevitably lead to differences in the NBS panel. Indeed, if NBS for CH and some inborn errors of metabolism were found to be cost-effective in countries from the northern hemisphere, screening for disorders as hemoglobin disorders and glucose-6 phosphate dehydrogenase deficiency may eventually prove to be cost-effective in sub-Saharian Africa, India and South-East Asia where these disorders are prevalent.

Finally, as proposed by Therrell and Padilla, there should be increased emphasis on point-of care (POC), taking advantage of newborn hearing screening and screening for critical congenital heart defects performed at the bedside [56]. Clear benefits should be achieved by the integration of these NBS modalities into a more comprehensive NBS infrastructure. Pilot projects should continue to be implemented in low-resource areas using a strategy that is integrated with current clinical care, possibly starting with hemoglobin disorders, with specialized expertise centered around one genetic service, and with the objective to evaluate the reduction of the genetic burden, cost-benefits, and barriers to implementation to help provide efficient NBS. However, it is unfortunate that the latest WHO recommendations on newborn health guidelines published in May 2017 did not identify NBS in the promotion of newborn health [57]. Progresses in the implementation of NBS in developing countries worldwide was thoroughly reviewed recently [56].

4. Conclusion and Perspectives

NBS is considered as a major public health success of the 20th century. NBS performed during the 1st few days of life through blood spot sampling on a filter paper followed by blood testing can prevent morbidity and mortality from severe conditions. NBS programs evolved from screening for one or a few disorders to potentially more than 50 conditions, thanks to the enhanced capacity of mass spectrometry to identify simultaneously multiple disorders with high sensitivity from only one sample injection into the instrument. However, there is still significant heterogeneity in the NBS panel offered by the different jurisdictions, in part due to variations in population genetics, but also in the various approaches and interpretation of the WHO screening criteria and decision-making process in the face of scarce data on disorders that are individually rare.

Following the wave of large-scale NBS expansion around the 2000's in many jurisdictions triggered by the introduction of MSMS, the implementation of high throughput NGS, which has already started to transform clinical genetics, has the potential to bring the NBS area to another level. Used on a large-scale, whole genome sequencing will identify another set of rare neonatal but devastating disorders for which presymptomatic interventions are available. It also has the potential to prevent misdiagnosis, shorten delay in diagnosis, facilitate early intervention, and improve diagnosis. However, there is a scarcity of data on most IEM, which may have highly variable clinical manifestations. The incorporation of NGS technology in NBS panels will need a stringent decision process that will consider evidence-based reliable methods from large-scale studies. Importantly, ethical concerns related to the generation of genomic information, a large proportion of which we still don't understand the clinical significance, will need to be addressed. As "too much screening is bad screening", the balance between benefit and harm must still be taken into consideration when evaluating the capacity of NGS to improve child's care. It should not be damped by precipitous, lax, and potentially harmful decision processes.

Finally, while about two-thirds of infants worldwide are born in limited-resource areas that still do not have any NBS infrastructure, several initiatives worldwide show promises, but are also faced with many barriers of implementation and sustainability. It would be a pity to come to the realization that the northern hemisphere continues to apply technological advances for the benefit of neonates and children, and the population at large, while low-resource areas are still struggling to implement a basic NBS infrastructure.

Author Contributions

YG designed the manuscript plan. YG and DB wrote the initial version of the manuscript and agree with its final version.

Competing Interests

The authors declare that they have no competing interests.

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