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Newborn Screening of Rare Diseases: Challenges in the Genomic Era

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Porto, maio de 2024

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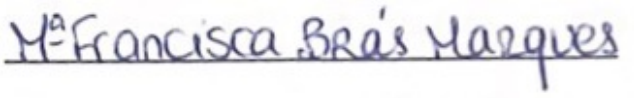

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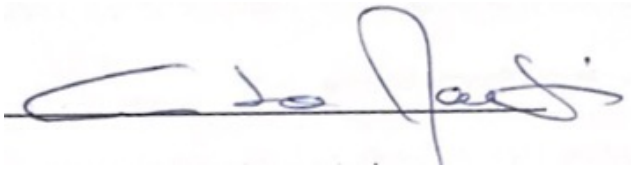

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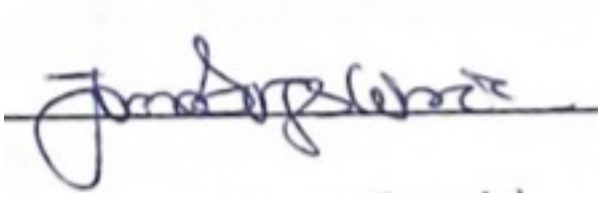

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Abbreviation List – Lista de Abreviaturas

CNV - Copy Number Variants
DBS - Dried Blood Spots
GS - Genetic Screening
IEM - Inborn Errors of Metabolism
MS/MS – Tandem Mass Spectrometry
NB – Newborns
NBGS - Newborn Genetic Screening
NBS – Newborn Screening
NGS - Next-generation sequencing
PKU - Phenylketonuria
RD - Rare diseases
SCID - Severe Combined Immunodeficiency
SMA - Spinal Muscular Atrophy
SNV - Single Nucleotide Variants
tNBS - Traditional Newborn Screening
VUS - Variants of Uncertain Significance
WES - Whole Exome Sequencing
WGS - Whole Genome Sequencing

Resumo

Introdução: O rastreio neonatal endócrino-metabólico, enquanto programa de saúde pública, é de grande importância pois visa detetar doenças potencialmente tratáveis em recém-nascidos antes do aparecimento de sintomatologia, reduzindo as taxas de morbilidade e mortalidade. Atualmente, assim como à data da sua criação, o rastreio tem sido feito com amostras de sangue seco em papel, tendo evoluído para o uso de tecnologias como a espectrometria de massa em *tandem*, alargando-se ao rastreio de várias doenças metabólicas e endócrinas. Dado que muitas doenças raras afetam doentes em idade pediátrica e que o seu diagnóstico é bastante demorado, a relação entre o rastreio neonatal e as doenças raras é crucial. Tendo em conta os avanços que se registam nos testes genéticos e nos novos métodos aplicados, como a *Next-Generation Sequencing*, a expansão do rastreio neonatal para incluir novas doenças parece ser inevitável. No entanto, existem considerações éticas, como o consentimento informado e o potencial de discriminação que devem ser tidas em conta. Além disso, é relevante sublinhar a importância do apoio político e governamental para a sustentabilidade e para uma vigilância eficaz do programa.

Objetivo: O nosso objetivo foi realizar uma revisão sistemática da literatura disponível sobre o futuro do rastreio neonatal de doenças raras. Este artigo destaca as oportunidades associadas aos avanços mais recentes nesta área, bem como os desafios que colocam.

Métodos: Foi realizada uma revisão sistemática da literatura sobre o rastreio neonatal de doenças raras utilizando as plataformas *PubMed*, *Scopus*, *Google Scholar*, *Mendeley* e *Science Direct*, publicada nos últimos 5 anos, em inglês e sem restrições em termos de país.
Foram utilizados dois critérios de inclusão principais: artigos publicados nos últimos 5 anos que incluíssem o rastreio genético de doenças raras e com tratamento atualmente disponível. Foram excluídos estudos cujo foco consistia em doenças raras que não possuem tratamento na data de publicação, rastreio de doenças frequentes ou fora do período neonatal. Foi elaborado um fluxograma PRISMA da pesquisa, que inclui abordagens dos critérios de elegibilidade e inclusão de estudos para a revisão sistemática.

Resultados: Após esta revisão sistemática, foram incluídos 50 artigos que abordam temas essenciais como a definição de doenças raras e a história do rastreio neonatal. Ademais, na área da genómica, são expostas as diversas técnicas de sequenciação genética e as oportunidades e desafios apresentados pela sua utilização num rastreio neonatal genético. Questões importantes como ética, eficiência, impacto nas famílias e opinião do público em geral e da comunidade médica, são também debatidos. Finalmente, sugerem-se abordagens e ideias específicas para implementar um possível rastreio neonatal genético, num futuro próximo.

Conclusões: A *Next-Generation Sequencing* visa otimizar o Rastreio Neonatal, seja como um teste complementar para melhorar os protocolos atuais, ou como método de primeira linha para identificar doenças que não possuem marcadores bioquímicos. No entanto, a sequenciação genética ainda não alcançou, na totalidade, a especificidade e os resultados céleres apresentados pela espectrometria de massa em *tandem* na deteção de muitas doenças hereditárias do metabolismo.

Palavras-chave: Doenças raras; Rastreio neonatal; *Whole Genome Sequencing*; *Exome sequencing*.

Abstract

Background: The systematic newborn screening as a public health program is of great importance as it is aimed at detecting treatable diseases in newborns before symptoms appear, thereby reducing morbidity and mortality rates. Originally using dried blood spots, the method has evolved with technologies like tandem mass spectrometry, expanding to screen for various metabolic and endocrine diseases. Given that many rare diseases affect children and diagnosis often takes years, the relationship between newborn screening and rare diseases is crucial. With advancements in genetic testing and technologies like next-generation sequencing, the expansion of newborn screening to include new diseases is inevitable. However, there are ethical considerations, such as informed consent and the potential for discrimination that need to be approached. Furthermore, it is relevant to emphasize the importance of political and governmental support for sustainability and effective surveillance of this program.

Purpose: Our aim was to conduct a systematic review of the available literature regarding the future of newborn screening of rare diseases. This article highlights the opportunities associated with the most recent advances in this area, as well as the challenges they pose.

Methods: A systematic review of the literature on newborn screening of rare diseases was conducted using *PubMed*, *Scopus*, *Google Scholar*, *Mendeley* and *Science Direct*, published over the last 5 years, in English and with no restrictions placed on country.
Two main inclusion criteria were used: articles published over the last 5 years that include rare diseases genetic screening with a current available treatment. Studies that focus only on rare diseases that have no treatment at the date of publication, screening of frequent diseases or not in the newborn period were excluded. A PRISMA Flowchart of the search was elaborated, including the eligibility criteria approaches and study inclusion for the systematic review.

Results: In this systematic review, 50 papers were included, covering essential issues such as the definition of rare diseases and the history of newborn screening. Furthermore, in genomics, the different genetic sequencing techniques and the opportunities and challenges presented by its use in newborn genetic screening are exposed. Important issues such as ethics, cost-efficiency, impact on families and the public and medical community point of view on this topic are also debated. Finally, specific approaches and ideas are suggested for the implementation of a newborn genetic screening in the near future.

Conclusions: Next-Generation Sequencing aims to enhance Newborn Screening either as a supplementary test to improve current protocols or as the first line method for identifying disorders that lack biochemical markers. However, genetic sequencing cannot yet replace the specificity and quick results provided by conventional tandem mass spectrometry in detecting many inborn errors of metabolism.

Keywords: Rare diseases; Newborn screening; Whole Genome Sequencing; Exome sequencing.

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1. INTRODUCTION

Systematic newborn screening (NBS) is a public health program, acting as secondary prevention, which aims to detect, in a pre-symptomatic state, diseases for which there is an available treatment, thus reducing their morbidity and mortality rates. It is one of the largest preventive medicine programs in western countries, complying with civic values and ethical principles that assure universal and equitable access to all newborns (NB) and the informed participation of parents. It is imperative that this program has political and governmental support to guarantee its sustainability and adequate surveillance, treatment, and follow-up of affected individuals[1-3].

The method, which is still in force, using a dried blood spot (DBS), was introduced in the 1960s by Robert Guthrie to screen phenylketonuria (PKU), but its success and effectiveness, as well as the discovery of new technologies, such as Tandem Mass Spectrometry (MS/MS) that enable a rapid, reliable and low-cost biochemical diagnosis, have allowed it to be extended to other metabolic and endocrine diseases[4-6]. This progress has always kept in mind the basic principles defined by the World Health Organization in 1975 (Wilson and Jungner criteria), that the disease screened must always possess a specific treatment, which should be started as early as possible, to avoid irreversible late sequelae or fatal consequences that often accompany the first episode of decompensation[7-9].

Nowadays, 38 million babies are offered NBS yearly, approximately 30% of those born each year on a worldwide scale. In recent years, the introduction of molecular screening technologies for pathologies such as Spinal Muscular Atrophy (SMA) and Severe Combined Immunodeficiency (SCID) has created the possibility to track rare diseases (RD) without a biochemical marker but with an available treatment[10].

In the present, we have acknowledged 6000 to 8000 RD, affecting the lives of 300 to 350 million people across the globe[10]. The average time taken to diagnose these pathologies is 8 years, placing a heavy burden on those living with a rare disease (families, caregivers, doctors, and society). As a result, the relationship between NBS and RD is of extreme importance, as the majority (80%) of these diseases affect children[11].

At this time, it is a fact that the greater knowledge of RD, the emergence of new therapies (enzymes, gene therapy, etc.) and access to new molecular diagnostic technologies, such as next-generation sequencing (NGS), through whole exome sequencing (WES) and whole genome sequencing (WGS), that show the potential of screening at a lower price per disease, will make the expansion of NBS for new diseases inevitable[12, 13]. Therefore, the use of genetic tests in screening will arise. It's not possible to predict exactly when or how genomics will play an important part in NBS, but a lot of progress in this area is taking place right now.

There are multiple uncertainties presented at this moment, both regarding which NGS technique should be chosen and the diseases, genes, and variants to be selected. There are also several ethical questions, namely: what an appropriate informed consent is or how to approach treatable late-onset diseases. The legal and social implications and the potential for discrimination due to misuse of genome data must also be considered[14-16].

We are at the beginning of a journey that will certainly change the horizon for NBS and several studies are being carried out to evidence the opportunities in using genomics in NBS.

2. METHODS

Protocol and Registration

The systematic review protocol was conducted in compliance with the PRISMA guidelines (Figure 1).

Search Strategy

Papers evaluating the new opportunities and challenges of NBS of RD in the genomic era were included in this systematic review. This search was conducted on five different bibliographic databases: *PubMed*, *Scopus*, *Science Direct*, *Google Scholar* and *Mendeley*, between January - March 2024. Authors used 3 different search combinations of the chosen MeSH terms for more precision and better results: firstly "Rare diseases; Newborn screening; Whole Genome Sequencing", secondly "Rare diseases; Newborn screening; Exome Sequencing" and, at last, "Rare diseases; Newborn screening". The search criteria for each database can be consulted in Figure 1.

Selection Process and Criteria

Pre-established conditions for eligibility were papers published over the last 5 years, in English, that comprised genetic screening of rare diseases with a current available treatment. Studies conducted beyond the newborn period or that included screening of frequent diseases were excluded.

Records Screening

References were retrieved from the bibliographic databases and imported into an *EndNote* library. After duplicates were removed, references were analysed by two independent reviewers (MFBM, JBC) based on titles and abstracts to screen for the eligibility of each study. In some cases, a full text examination was required. A third member was available to solve any disagreements (EGM). In a second phase, the full text of the resulting records was assessed for study inclusion.

Quality Assessment

Since the majority of the retrieved studies were descriptive, no specific tools were applied to verify the quality of evidence of each paper.

Data Extraction, Synthesis and Analysis

From the studies included, a spreadsheet was created to summarize the following information: author(s), study name, reference, RD, NBS, WGS and WES, Opportunities, Challenges, Parental Point of View, Ethical Questions, Cost Effectiveness, Real World Examples, Future NBS. Information retrieval was reviewed by the other authors in a cross-over manner.

Data is described in the results section in a qualitative and narrative manner, focusing on the topics mentioned above.

3. RESULTS

a. Literature Search

The search identified 1298 papers (68 from PubMed, 140 from Scopus, 447 from Science Direct, 145 from Mendeley, and 498 from Google Scholar). After removal of 379 duplicates, a total of 919 papers were submitted for screening. By reading the titles and abstracts, 862 papers were excluded as they focused on children other than newborns, frequent diseases or rare diseases that have no treatment at the date of publication, resulting in 57 papers to be considered for inclusion.

A second screening to assess eligibility was performed after reading the full papers, which resulted in 7 additional papers being excluded, because they did not focus on newborn screening specifically (four), whole genome and exome sequencing (one), or we did not have access to them, and e-mails sent to authors were not answered (two).
This resulted in 50 papers to be included in the systematic review. Twenty-four of them were original/research papers, 14 literature reviews, 4 commentaries, 3 editorials, 3 opinion articles, 1 systematic review and 1 viewpoint article.

b. Rare Diseases

RD are defined by no more than 1 person affected in 2000 individuals. These conditions are present in 3,5% to 5,9% of the world population. Despite their rarity, they exert a significant collective influence on public health[17], and substantially contribute to infant mortality and permanent disability[18]. RD impose a heavy burden, often leading to lengthy diagnostic journeys, marked by misdiagnoses, unnecessary procedures, and ineffective treatments[10]. Moreover, they frequently necessitate expensive therapies beyond the financial means of individual patients[4]. For example, cutting-edge treatments like gene therapies and enzyme replacement therapies come with extremely high prices, contributing to the substantial economic expense of RD[17] of approximately 1 trillion dollars in the USA alone in 2019. Addressing these challenges requires early diagnosis, which not only benefits patients' psychological and social well-being but also their physical health[10].

RD exhibit a wide array of disorders and symptoms, differing not only between diseases but also among patients with the same condition. Approximately 75% of RD have a genetic basis, underlining the necessity of genetic diagnosis for disease comprehension[10]. However, RD pose significant obstacles to the inclusion of robust statistical data in NBS programs, given their complex diagnostic pathways and all the reasons mentioned above[9, 10].

Despite increasing awareness, early diagnosis, and effective treatment delivery remain global health challenges for the twenty first century. The majority of RD continue to be overlooked, under-researched, and undertreated, highlighting the urgency of addressing these disparities in healthcare.

c. Newborn Screening Over the Last Years

The origins of NBS (Figure 2) can be traced back to the breakthrough made by Horst Bickel in 1953 regarding the treatment of PKU, although its formal introduction occurred only in 1963 when Robert Guthrie pioneered its application for PKU detection using blood samples absorbed onto specialized collection paper[9]. In early years, NBS focused on inborn errors of metabolism (IEM), however, its span expanded over time. For instance, later in 1973, a technique used to

identify hemoglobinopathies from DBS was created, followed by the introduction of NBS for congenital hypothyroidism in the subsequent year. In 1987, a method for DNA extraction from DBS was created, facilitating its use as a second-tier test to diagnose sickle cell disease. In 1988, cystic fibrosis was added to the NBS panel, also consisting in DNA extraction from DBS[6].

The introduction of MS/MS, in the 1990's was the next major discovery in NBS[6, 9, 19], enabling a significant increase in the number of conditions screened in a cost-effective manner[19]. It has undoubtedly saved lives and further enhanced the efficiency and accuracy of NBS processes[6, 9]. This method has become the prime technique employed in NBS centres in developed countries to detect biochemical metabolites, associated with amino acid metabolism, organic acid metabolism, and fatty acid oxidation in DBS[20, 21]. Nevertheless, contemporary NBS with MS/MS poses some challenges. Namely, it is constrained to blood or urine-based metabolic biomarkers and many early-onset genetic conditions lack particular metabolic biomarkers with disease-specific interventions, resulting in their exclusion from systematic screening[22, 23]. Furthermore, MS/MS operates in alignment with Wilson and Jungner principles for NBS disease selection, however it may identify other disorders that do not fulfil these criteria[15, 18, 24].
The framework for selecting conditions for screening adheres to the principles outlined by Jungner and Wilson in 1975, which have been adapted over time to accommodate advancements in testing technologies. These principles are strong, though they admit flexibility to account for regional disease prevalence, ethical considerations, and further local factors[19]. The selection criteria for diseases to be tested in NBS programs initially encompassed several key factors: the potential for serious consequences or mortality, a relatively high incidence with well-defined pathogenesis and natural history, the ability to detect the disease despite being asymptomatic in an early stage through laboratory testing, the availability of accurate and reliable screening methods with acceptable false positive and false negative rates and costs, the presence of effective treatments that can improve outcomes with early intervention, a reasonable cost-benefit ratio, and the presence of actionable suggestions for diseases lacking effective treatment options[6, 15].

In Europe, each year, 85% to 100% of the 4.2 million NB undergo screening of up to 40 disorders, while in the USA, almost all of the roughly 3.7 million NB annually are subjected to NBS for more than 50 illnesses[23]. The number of disorders included in each screening panel can vary significantly both between and within countries[25]. Traditional NBS (tNBS) programs are also documented in other continents, for example North Africa, the Middle East, Latin America and the Asia Pacific. Non-profit benefactor organizations are actively contributing to the application of NBS programs in low resource settings[22].

In the United Kingdom, NBS is frequently considered standard care, and while parental authorization could technically be negative, it is typically not addressed since participation is generally assumed to be in the best interest of the baby. On the other hand, in many other countries like the USA, the rationale for testing in the baby's best interest is more explicitly stated, and NBS is compulsory with limited options to opt-out[14, 27]. After all, NBS plays a crucial role in enhancing quality of life for NB by facilitating early diagnosis, prevention, and treatment of IEM.

As for the process, typically, one or two samples of DBS are usually assembled between 72 and 168 hours after birth and analysed, as seen in countries like the Netherlands and Portugal[25, 26]. DBS, usually obtained by heel prick test, are preferred for sampling since healthy NB lack venous access, and these are vastly approved for use in NBS workflows[8].

To sum up, while conventional NBS methods offer simplicity and convenience, they are often limited caused by the influence of various factors on biomarkers or enzymatic activities[7]. For this

reason, numerous developed countries, including the USA, the UK, and China, have embarked on pilot genomic NBS studies, setting the example globally[22]. Disease-associated DNA molecules or genes analysis presents a promising supplementary technique for tNBS, especially for diseases lacking these specific biomarkers like SCID and SMA[7].

d. The Genomics Era

In the beginning, *The Human Genome Project*, an international scientific research endeavour initiated in 1990 and concluded in 2003, aimed to decipher the structure of human DNA, identify, map, and sequence all human genes, both in terms of physical structure and functionality. This project profoundly advanced our understanding of genetic disease mechanisms, uncovering new disease-associated genes and variations, which often manifested in unusual disease presentations[11]. Genetic sequencing has become an indispensable tool in healthcare, demonstrating its utility across various medical disciplines over the last few decades[29].

Despite the promise of Newborn Genetic Screening (NBGS) inherent in *The Human Genome Project*, practical implementation was delayed due to limitations in genomic knowledge, biotechnology, and informatic tools[1]. However, significant steps have since been taken, with genomic sequencing now routinely employed in clinical settings due to its speed, cost-effectiveness, and comprehensiveness compared to traditional methods, particularly in cases of RD[18]. The advancement of genomic sequencing techniques has facilitated the incorporation of genetic screening (GS), into routine clinical diagnostics, especially in neonatal intensive care units where it serves as a first-tier diagnostic test for infants suspected of inheriting IEM or other genetic conditions[26, 30].

Efforts to expand NBS with the use of sequencing technologies have intensified, driven by the prospect of transformative therapies. USA's Food and Drug Administration anticipates a surge by 2025 in gene and cell therapy approvals, hopefully followed by equivalent development of NBS methods adapted for precise gene variant identification[12].

Some techniques that may be applied to NBGS are Sanger sequencing, MassARRAY nucleic acid mass spectrometry, fluorescent quantitative PCR, and NGS technologies which include disease-targeted gene package panel sequencing, WES (sequencing of coding regions of all known genes) and WGS (sequencing of the whole genome)[11, 15, 19, 21, 31, 32]. Today, NGS is applied clinically on a regular basis as it is faster, cheaper, and with better comprehension comparing to other methods, such as Sanger sequencing[11]. Benefits of targeted NGS, particularly in case only a panel of known variants is analysed, include a decrease in the number of variants of uncertain significance (VUS) discovered and in the time needed for interpretation. Even so, clinical sensitivity can be quite low since we are merely targeting a known panel of variants[33]. This review will focus on the use of WGS and WES as methods of screening.

The integration of genomic sequencing into NBS programs represents a significant milestone in healthcare, offering unprecedented opportunities to identify and intervene in genetic diseases at their earliest stages[29].

e. Whole Genome Sequencing and Whole Exome Sequencing

On the one hand, WES is a type of NGS that specifically targets the exons of the genome and evaluates the adjacent intronic regions, typically 10-20 bases[11, 25]. WES covers no more than 1.5% of the dimension of the WGS, meaning faster and cheaper sequencing, while maintaining a considerable quantity of genomic data[21]. While WES accurately identifies single nucleotide variants (SNV) and indels up to 50 base-pairs, it may miss some small copy number variants

(CNV) and structural variants[18, 25]. Using this method, we capture specific regions of DNA and afterwards we enrich them for additional processing, possibly leading to false negative results[21]. In general, WES has elevated sequencing depth, yet is more biased in overall coverage, comparing to WGS[11].

On the other hand, WGS examines the entire DNA sequence of the genome, including coding and non-coding nuclear sequences, besides the mitochondrial sequence. It provides uniform coverage and can detect SNV, indels, non-coding variants (that can change gene expression, splicing, and chromosome replication), mitochondrial variants, CNV, and structural rearrangements[3, 6, 11, 18, 21, 25, 34]. WGS offers broader disorder detection compared to current NBS methods and may reduce false positives[33]. Notably, in some IEM, WGS has revealed large deletions undetected by WES alone[6]. WGS, being a non-targeted technique, allows for subsequent adaptation, since clinically relevant genes and regions may be included in reports without altering sample assemblage or laboratory workflows. Additionally, WGS libraries involve a quicker preparation than those obtained through enrichment strategies, ensuring faster sequencing. Overall, WGS seems to be a more thorough option for genomic analysis[25, 34], despite not being as fast and cheap as WES, in general.

f. Opportunities of a Newborn Genetic Screening

Transitioning from tNBS to NBGS aims to address some key goals: decreasing false positive and false negative rates, enhancing positive detection, and amplifying the range of diseases screened[10, 22]. Recent progress in innovative sequencing approaches, associated to the present capability to collect, store, process, and interpret enormous amounts of data, means we have come to a turning point that shows a way into a promising future for RD screening[10].

NGS is characterized by its high throughput, sensitivity, specificity, and a considerably lower cost of single base extension in comparison with first-generation sequencing[7, 35, 33, 36]. It enables preliminary diagnoses in about a day, reducing turnaround time compared to traditional methods, which in some cases could take up to 2 months to obtain results[7, 22, 35]. In spite of disease heterogeneity, numerous IEM have in common an identifiable genetic target, which indicates that a NGS-based NBS is a logical solution, allowing the screening for hundreds of disorders to be made at the same time by a single platform[33, 37]. The fact that it is more straightforward to incorporate a disorder in screening merely by adding a gene to a genetic panel than by including a biochemical test in a biochemical panel is a benefit that should be underlined[26].

Another advantage consists in the fact that a specimen for genomic testing can be collected immediately following childbirth as, in this modality, it is unnecessary to wait for an atypical metabolite to spread for 1–2 days as demanded by the present biomarker screening[25].

Moreover, NGS has potential applications in pharmacogenomics and personalized and precision medicine, offering greater insights into disease physiology and aiding in the identification of actionable variants[16]. This resource also aids in clarification of ambiguous screening results[33, 36].

There are various studies demonstrating the effectiveness of NGS-based screening approaches. Armstrong *et al*.[27] mentioned a study differentiating results from WES and tNBS that discovered these two techniques gave complementary information. In 9.4% of sequenced NB, WES identified genetic risk for conditions not detected through standard NBS. The feasibility of a first tier NBGS approach has been corroborated by Kingsmore *et al*.[37], who reported 388 clinically actionable diseases in 2208 severely ill NB in Intensive Care Units with 99.7% specificity and 88.8% sensitivity. On the other hand, Veldman *et al*.[26] presented studies in which WES was

carried out to confirm diagnosed IEM expected a bright future for NGS-based NBS, as they anticipate a decrease in false positive results, especially regarding cystic fibrosis screening.

g. Challenges of a Newborn Genetic Screening

ii. Next Generation Sequencing

When integrating a new technology into a state-operated program, achieving clarity in assessing its benefits and limitations is crucial, especially with GS, given the varied, intricate, and uncertain nature of test outcomes[38]. Firstly, one primary concern revolves around the speed of preparing, analysing, interpreting, and reporting results to meet the requirements of NBS, efficiently[21]. The high cost of NGS, coupled with limitations in variant detection (such as intronic variants, regulatory regions, CNV, structural variants, and trinucleotide repeats), as well as challenges in variant interpretation and access to annotated disease databases, pose noteworthy barriers to using DNA as a primary screening tool.
The difficulty in interpreting variants without a clear phenotype, makes it challenging to assess the pathogenicity of certain variants, thus diminishing the clinical utility of reporting some variants for screening purposes. Furthermore, RD lack a comprehensive understanding of the full range of the phenotype[21, 25]. Also, the sheer volume of samples sequenced with NBGS is expected to uncover many more VUS, complicating interpretation further[21]. This persists as the main limitation of WES and WGS implementation in NBS, as it may lead to ambiguous, misleading results and a longer turnaround time[6, 7, 11, 17, 18, 20, 21, 26, 32, 35, 36, 39, 40, 41, 42].

Ethnic diversity also poses a challenge in database representation, potentially leading to misinterpretation of VUS. Therefore, building highly representative genomic population databases is essential for accurate variant calling and interpretation across different ethnic groups[2, 9, 21, 37, 41, 42].

On the other hand, a NBGS panel may uniquely cover pre-selected known mutations, so it can miss some pathogenic variants, causing false negative results and decreased detection rates[7]. This means the creation of a target gene library is highly relevant for the whole NGS process, as its quality will influence the precision of future sequencing data[35]. However, creating standard protocols for NGS library preparation is quite laborious and some authors say it could not be used in a NBS time frame[21].

The possibility of missing disorders that need biochemical recognition, such as congenital hypothyroidism, is another factor that may impede its implementation. Therefore, the necessity to combine tNBS results with NGS results must not be ignored[16, 32].

Additionally, a major limitation of present investigations consists in the fact that they have mainly been carried out in a different context, instead of a screening laboratory that, depending on birth rate, demands both low and high throughput capabilities[2].

iii. Public and Medical Community Acceptance

Nowadays, approximately 98% of NB participate in NBS, in the USA[1]. However, maintaining this high participation rate poses challenges, especially with the need for parental consent and the use of genomic data. The introduction of GS technology has fundamentally changed how genomic information is communicated, challenging traditional methods and public expectations[16].

Individual factors influencing adoption of this method include recognized relevance to an individual's health or family, previous participation in screening and healthcare, resources to access and understand information, engagement from trusted sources, and personal values[43].

Concerning health systems, factors such as organizational culture, leadership, education and training access, and collaboration capacity across specialties influence the adoption of NBGS. Societal acceptance and trustworthy systems are crucial, particularly in public funded national health systems[43].

Disbelief in medical practice has an impact that should not be overlooked, as, in recent years, it has been intensely corroborated by the COVID-19 pandemic. Scepticism, misinformation, and uncertainty, mainly among ethnic minorities, individuals of lower socioeconomic status or levels of education caused a decrease in uptake of vaccinations[5]. Public trust in genomic research requires transparent practices, communication allied to genomic health education, and community participation[39]. Media coverage can raise expectations but also evoke fears, potentially impacting public acceptability of NBS[16].

Some studies have been developed to explore what the opinions of health professionals, parents, etc. are on the application of NGS to NBS. Firstly, a cross-sectional survey was developed by Wu *et al.*[44] in China to recognize the knowledge and compliance of healthcare professionals who work in NBS centres. Among 258 participants, 81% answered that they were interested in NGS, and 69% were aware of it. Moreover, 98% of the individuals inquired believed in the relevance of the application of NGS-based NBS in China, yet 5 participants considered it unnecessary. Seventy three percent of participants were concerned about the incapacity to give genetic counselling at the present time, considering it would be the major obstacle to application to clinical practice. In addition, 94% of healthcare professionals were convicted that NGS should go abide by precise screening principles for disorders and pathogenic genes and proposed a few serious genetic diseases with high incidence and a possibility for intervention or treatment which could be included in the screening. Professionals with greater institutional and personal education level were more involved towards NGS and were clearer about the technology selection. Nevertheless, their risk awareness was higher as well. In another study, designed by Gold *et al.*[13], younger experts tended to believe that GS for treatable genetic conditions that are not yet included in USA's Recommended Uniform Screening Panel should be available for all NB, with highest concordance among metabolic and endocrinologic disorders. The introduction of further treatable, infant-onset metabolic conditions that do not have a specific biochemical screening biomarker and that could easily be analysed on a population level was also vastly accepted. As well as the addition of diseases with ground-breaking emerging pharmacologic therapies, such as acid sphingomyelinase deficiency and Duchenne muscular dystrophy. Finally, Clark *et al.*[5] focused on the perspectives of health professionals and parents. The first group included in this study vastly supported the targeted use of WGS, focusing on clinically actionable childhood-onset conditions, and enhancing the sensitivity and specificity of tNBS. They favoured the use of WGS for critically/chronically ill and high-risk NB/children, rather than its application for healthy infants. Differences between work carriers were acknowledged, as genetics-specialists were less supportive of WGS-NBS and the use of WGS more widely, compared with non-genetics health professionals. This was specifically noticeable where results dealt with untreatable disorders, non-serious disorders, risk/predispositions, and VUS. Public in general often presented issues related to infrastructures and the possibility of adapting them to the implementation of WGS. Other matters were discussed, such as the possibility of a loss of privacy, including concerns with data security, financial exploitation by pharmaceutical businesses and employment or health/life insurance discrimination. Some were afraid that the immense volume of information, associated with an ambiguity of findings could cause

uncertainty and anxiety with future parental frustration or mistrust, resulting in a reduction of NBS uptake.

iv. Newborns and their Families

Families are crucial in implementing NBGS, and their concerns and viewpoints must be valued for the future success of NBGS and maintaining parent trust[27]. Data from NBGS can raise issues like damaging self-esteem, stigma, parental guilt about passing on genetic variants, discrimination, and privacy worries[3, 25, 38, 40, 45]. Uncertain results might label healthy NB as "pre-sick" or "patient-in-waiting", leading to unnecessary medicalization and distress for parents[2, 14, 16, 21, 25, 32, 38, 41, 43, 46]. In addition, false positive results can cause anxiety and the need for further testing[17, 21, 43].

Nowadays, NBS programmes operate on an opt-out system, which means testing proceeds normally unless NB families decline it[21, 37]. In surveys, parents of healthy NB were more supportive of tNBS than NBGS and many believe parental consent should be required for NBGS[27, 38]. However, screening is often viewed as routine rather than a choice for parents[14]. While parents typically make health decisions for their children, in many countries, governments may intervene if decisions contradict the child's best medical interests[37]. Furthermore, decisions about which results to disclose to parents should be made carefully, considering potential psychosocial effects, namely overwhelm, postpartum stress, the absence of "normal" time with the child while in an asymptomatic period, and bonding difficulties[5, 40, 41, 45]. Informed Consent and Results Disclosure will be further explored in "Ethical issues".

In a study developed by Clark *et al*.[5], parents of children with RD seemed keener on the idea of a NBGS implementation for future children than other parents. However, they focused their attention on the need to consider both the potential benefits of reaching a diagnosis and creating increased uncertainty through equivocal results.

v. Ethical Issues

GS presents ethical, social, and legal challenges due to the richness and personal nature of genetic information[1, 37]. These issues are often compared to those associated with tNBS, as many have not been encountered in practice yet[1]. A comprehensive system of guarantees and safeguards must be established by law to protect the rights of individuals involved. These encompass rights to privacy, personal image, and dignity, as well as the freedom to choose whether to receive genetic information or not. Moreover, rights to health and bodily integrity are paramount. Notably, the principle of the best interests of the child holds significant importance, particularly regarding genetic information concerning minors or directly affecting them[15].

iv.1. Selection of Diseases to be included in NBGS

Wilson and Jungner original screening criteria, focusing on early detection and treatment availability, have been re-evaluated considering genomic advancements, medical technology evolution and increased interest in GS[28, 38]. The selection of diseases for screening now considers factors such as genetic feasibility, sensitivity, specificity, and predictive positive value[25]. Disease selection prioritizes monogenic disorders with well-established genetic biomarkers and genotype-phenotype correlations[16, 35]. To ensure uniform care and program assessment, it is recommended to develop case definitions, which are sets of standard criteria used to analyse whether a child has a certain genetic disorder and should consequently be treated[25]. The scope

of the gene list can vary depending on program goals, ranging from a small initial selection with extremely relevant genes to a more extensive list[40].
NBS focuses on serious illnesses with available interventions[15], while NBGS may be considered for conditions without direct medical treatment if there are benefits to the baby, family support and reproductive decisions, and societal knowledge about the disease[9, 16, 28, 38]. Additionally, outcomes of treated cases during clinical trials inform the appropriateness of conditions for screening[9].
Equitable access to interventions is also a crucial consideration in disease selection. This implies input from health systems and other relevant clinical entities[16].

iv.2. Insurance Issues and Healthcare Access

Another emerging concern involves the potential repercussions of NBGS results on dealings with insurance agencies, including income protection and life insurance, where a genetic diagnosis of a rare disease could affect access to health insurance and may lead to discrimination, possibly resulting in a shortened lifespan[2, 3, 32, 37, 38]. Expanding NBGS to cover more diseases requires ensuring ongoing healthcare access for all children, not just those with a definite diagnosis[2, 11, 23]. In addition, it should be noted that overburdening genetic laboratories with screening healthy NB could lead to delay in testing for sick babies, potentially worsening existing service pressures and failing to benefit those with rare conditions, as intended in the first place[14].

iv.3. Discrimination

Protecting children is a moral imperative due to their inherent vulnerability and the profound impact of poor health and disease on their development and well-being[2]. However, the political and social stability of a country influences the feasibility of expanding NBS and addressing key healthcare issues. Nations struggling with childhood mortality from malnutrition may prioritise other health concerns over NBS expansion[4].
Consequently, ensuring equitable access to GS remains a significant challenge. This disparity risks exacerbating existing health inequalities and may subject participants to direct discrimination, including limitations in education or employment opportunities stemming from future data re-analysis and disclosure[4, 17, 39, 43].
Studies conducted in the US have revealed that ethnic minority individuals harbour more pronounced worries regarding genetic testing, such as suspicions about data storage and reuse and fear that results could perpetuate racial discrimination[5]. The underrepresentation of ethnic minority participants in genomic datasets further compounds these inequalities, resulting in limited and unrepresentative treatments and knowledge in genomic medicine for these populations[39].

iv.4. Informed Consent

Genetics professionals surveyed by Ulm *et al.* advocate for a new counselling paradigm for GS in NBS, emphasizing non-mandatory programs with consent and opt-out options to prevent genetic discrimination[3, 38, 45]. In fact, empowering parents to make informed decisions about participating in NBGS is essential[14, 45]. Before specimen collection and testing, guardians of NB sign an informed consent, after being fully briefed on the screening's purpose, capabilities, limitations, and management of expectations[15], as NBGS also implicates the genetic information of the newborn's biological parents and siblings[14].
However, formalized parental consent processes may result in fewer babies undergoing screening, highlighting a potential cost of introducing such screening[14].
Persistent concerns may impede broad parental consent, particularly among underserved populations[45].

Regarding the time frame in which the information should be given to parents, there are different opinions presented in the literature. While antepartum enrolment allows for thoughtful decision-making, scaling this approach is logistically challenging, given the widespread availability of prenatal care compared to delivery suites. Additionally, the materiality of NGS-based NBS may not be apparent until after birth[8]. Some argue that the postpartum period isn't conducive to making decisions with lifelong consequences[14, 39]. Bros-Facer *et al.*[23] described one initiative of NBS programmes that evaluated varying the timing of informed consent. Participating centres were offered the flexibility to adapt their timing to their choice. A tiered approach to consent, providing information during pregnancy followed by reminders after birth, could be effective[39]. Alternatively, online consent modules offer opportunities for comprehensive education and addressing questions[25].

iv.5. Results Disclosure

Returning results to patients from WGS studies in clinical practice or research, is widely acknowledged as a principle. Nonetheless, electing the findings to be disclosed to families is a crucial step for any screening or research program. There is ongoing debate regarding this issue, as for what is best practice, and the responsibility this sets upon researchers to search for and share findings[39]. Having in mind both the rights and needs of the child and the interests of the newborn's family when disclosing results is crucial.

Generally, there are four categories of results that could be disclosed in NBGS programs: childhood-onset actionable conditions, childhood-onset non-actionable conditions, adult-onset actionable conditions, and adult-onset non-actionable conditions. Returning childhood-onset actionable conditions is the only non-questionable category generally recommended for NBS programs[39, 40]. Genes reported should ensure sufficient evidence demonstrating their association with childhood disorders with high penetrance. Also, genes with moderate evidence or penetrance, where intervention in childhood has an important role in the prevention of subsequent major diseases, should also be considered for the results disclosure. In general, only variants with pathogenic or suspected pathogenic significance should be reported, to avoid confusion and complexity in clinical consultation. In cases where only one pathogenic or likely pathogenic locus is recognized in an autosomal recessive genopathy, it should be communicated as a likely carrier, with a recommendation for follow-up diagnostic testing in case the gene-associated phenotype is consonant with other clinical indications or family history. Loci causing mild symptoms or late onset may not be reported initially but could be considered after comprehensive evaluation of family history and other clinical indications. Additionally, variants with implications for female heterozygous carriers of X-linked gene disease-causing loci should be reported, considering the potential for non-classical phenotypes caused by X chromosome inactivation preference[15, 35].

Some view returning adult-onset conditions as being in the newborn's best interests, even if it disregards their right to an open future, particularly if the results are relevant to imminent clinical care. Others claim that it could still be in their best interest if the findings benefit the child's parents by informing them of their disease risk. In opposition to this argument, some say that best interests should focus on the child and, in case the parents' health is at stake, then they should be tested directly[40, 46]. Furthermore, it is imperative to remember that many legal acts across the globe acknowledge the "right not to know" as a fundamental patient's right[21].

iv.6. Data Storage

Long-term storage of genomic data is a crucial part of this process. Data could be claimed at various stages in life, which means DNA and data storage should be kept under exceptional protection. Legal regulations should be required on who has access to this data to prevent legal handicaps[3, 20, 21, 35, 40, 41].

Some authors mention that the most ethical and secure approach, with the highest guarantee, involves destroying genetic information post-analysis, retaining solely the list of screened disorders provided to individuals and their parents. If, in the future, the individual requires genetic information upon reaching adulthood, this could be easily obtained directly from a biological sample, based on an informed decision[3, 15]. Nonetheless, there are potential advantages of retaining genetic information in the long run, including future discoveries of new genes and advancements in understanding the pathogenicity of genetic variations. Furthermore, technological progress may enhance diagnostic capabilities[9, 37].
In the UK, for example, privacy and data sharing regulations closely align with the European General Data Protection Regulation. As a matter of fact, participants in NBGS programs have the right to access their data, and the NHS Constitution for England stipulates patients' rights to be informed about data usage and decide on its sharing for research purposes[39].
Revisiting genomic data in the future will become a reality, in consequence of discoveries of better screening algorithms and the appearance of new treatments. Therefore, clear expectations regarding recontacting families after the initial screening result should be established[25].

vi. Cost Effectiveness

Evaluating the cost-effectiveness of NBGS is crucial, given its reliance on public funds. It must demonstrate at least comparable cost-effectiveness to current NBS programs, although significant uncertainties persist[39], as economic analysis becomes particularly complicated when NBGS overlaps with existing screening programs[25]. The complexity of hereditary conditions and their vast consequences also contribute to the difficulty in developing comprehensive economic models[17]. Consequently, there is little information regarding the cost-effectiveness of using NGS in NBS[1, 17]. Extensive assessment of health economic data and cost-benefit analyses for NBGS, as well as the costs of not implementing it, are imperative[26, 29, 37]. This evaluation should encompass the costs of NGS, infrastructural requirements, healthcare provider involvement, laboratory personnel and clinical follow-up and treatments or prevention programs[3, 6, 9, 15, 37]. The potential lives saved, quality life years gained and the impact of genetic information on quality of life should also be taken into consideration[37, 40].

While some authors mention NBGS is intended to complement rather than replace tNBS, the current cost of tNBS provides a benchmark for what is acceptable for public funded NBGS[1]. Costs for tNBS typically range from €1 (screening for 2 conditions in Moldova) to €43.24 (screening for 17 conditions in the Netherlands) per NB in Europe and approximately $100 per NB in the USA (more than 50 conditions screened). In Israel, screening for 12 diseases costs around $45 per sample[21,29]. In contrast, diagnostic WGS can cost $8000-8500 per NB, although the interpretation burden of NBGS is significantly lower. With advancements in technology, the cost of WGS is projected to decrease to $100-200 per NB in the near future, provided it can be performed at a massive scale and with near-complete automation[1,8]. Van Campen *et al*.[33] developed a study in UK's NHS in which the total cost of an assay would be approximately £71.14/sample. Taking into consideration the cost per sample (approximately £25 per baby) nowadays, they concluded the NGS assay developed is unexpected to be considered cost effective as a first line NBS test in the UK, as it is.

Once the bioinformatics pipeline is established, the price of NGS and its analysis for 10 or 500 genes is practically the same[25]. This cost (the price of sequencing for each million bases) has decreased greatly[20, 21, 26], and some say WES is less expensive than WGS[25]. Projects such as *NSIGHT1* and *Project Baby Bear* revealed that GS, as a first-line test, decreases healthcare costs by $6,000 to 15,000 per child and between $1 - 3 million per health system and may be cost-

neutral or cost-saving, by preventing severe symptoms that require frequent hospitalizations[23, 38].

However, implementing NBGS poses challenges, including the need for numerous high-throughput next-generation sequencers and increased manpower for interpretation, which may exceed the capacity of some healthcare systems[21]. Moreover, the cost of NBGS testing may delay its development in countries with low-income and decreased literacy rates[32]. Another complexity arises when diagnosing RD, as financially feasible therapies may be scarce, posing ethical dilemmas regarding access to treatments. Additionally, drugs to treat these diseases may have limited development and production due to lack of profitability, especially in resource-limited settings[4].

Despite the expenses associated with lifelong therapies for identified individuals, cost-benefit analyses have shown that the final cost is less than the financial load of a shattered person, and causing an impact on the individual, their family and the community[4]. Further evaluation and modernization of NBS financing programs are necessary to guarantee equitable access to services and treatments, keeping pace with improvements in the field[9].

h. Examples

The landscape of NBGS has seen significant advancements and explorations through various global projects since 2013. All these initiatives aim to assess the clinical utility, feasibility, risks, benefits, and costs associated with integrating WGS and WES into NBS programs[22, 23, 18, 42].

The *BabySeq Project* is an American randomized test on 159 NB (127 healthy participants; 32 registered from intensive care units). It proved the efficacy of NBGS in detecting risk and carrier status for a broad variety of disorders not detectable by current NB assays[14, 38]. Results revealed childhood-onset disease risk in 9.4% of NB and carrier status for recessive diseases in 88%[23].
The *North Carolina Newborn Exome Sequencing for Universal Screening* (*NC NEXUS*) project was concluded in 2020 and evaluated 106 babies. A 466 gene panel efficiently identified 88% of the cases with already diagnosed IEM. Additionally, actionable findings that otherwise would not have been discovered by tNBS were reported in four NB. While it found that WES could not entirely replace current screening tests, it showed that genomic information could enhance the sensitivity and specificity of NBS for metabolic disorders[14, 38].
NGSf4NBS Project is a technical feasibility study created in the Netherlands that focused on ethical, legal, social, and financial features of NGS as a first-tier method in NBS[38].
BeginNGS Consortium is a new international consortium that suggests the implementation of a self-learning healthcare delivery system for NBS for hundreds of genetic disorders, diagnosis verification, adequate treatment application, and drug development acceleration. It began with the idea that diagnostic WGS required a few changes for WGS-based NBS[8].
NB-Seq Project analysed WES as a method for NBGS[18, 40], demonstrating the potential to reduce false positive results and facilitate timely case resolution[47], although they concluded that NBGS is not yet prepared to be a primary NBS test for all IEM[18].
The *Screen4Care* initiative focuses on shortening the path to diagnosis for people with RD through many initiatives, including a NBGS. Some reported benefits of its implementation were a better patient journey, healthcare upgrade, better quality of life, reduced irreversible harm and informed reproductive decisions for NB and families. Various advantages on a Healthcare perspective were explored, such as a paradigm change in RD screening and diagnosis, acquiring better disease epidemiological and natural history data, care delivery and integrated care among different specialties, that will be beneficial in the long run[10, 25].
The *Newborn Sequencing in Genomic Medicine and Public Health* (*NSIGHT*) network was funded by National Institutes of Health in the early 2010's[48]. This project addresses key research

questions related to DNA sequencing and newborn health, aiming to replicate or augment known NBS results, explore conditions not currently screened for, and provide additional clinical information relevant to newborn care[6]. They found that NBS by WES was less sensitive for RD than MS-based NBS: WES had 88% sensitivity for these disorders in 691 true positive samples by MS-based NBS[8].

After all, these projects collectively demonstrate the potential of a NBGS to detect an extended variety of diseases and specific variants and affirm the real value of GS[32], as well as their limitations, without discarding the need for further studies.

4. DISCUSSION

Having all the points already stated into consideration, and the variety of studies made regarding the implementation of NGS in NBS, it is time to start designing a future model for the NBGS. This requires comprehensive collaboration among various stakeholders, including clinicians, researchers, ethicists, public health professionals, policy makers, and patient representatives[40]. Key factors that may influence the implementation of NGS in NBS will probably mirror those affecting the uptake of other population screening programs, genomic testing and innovating technologies[43].

It remains uncertain whether first tier NGS-based screening will supplant existing technologies, be used in areas where current methodologies are lacking (for diseases without an existing MS/MS platform) or be employed concurrently. Additionally, there is a question of whether these approaches should vary for specific diseases to enhance diagnostic effectiveness[37]. Many authors suggest that GS will run in parallel with tNBS, a widely trusted population screening program, up to the time that the sensitivity and specificity of NGS-based screening match those of biochemical screening for all diseases covered in NBS programs[3, 7, 23, 49]. On the other hand, a two-stage NBS process has been proposed, involving biochemical testing for conditions necessitating immediate diagnosis in the newborn period, alongside molecular genetic testing for conditions requiring less urgent treatment[19]. Some anticipate that NBGS will become the primary NBS test within the next decade to fifteen years[23].

The importance of transparency in opening the door for public trust is often emphasised, with information sharing seen as a primary method[9, 39]. Information must not only be accessible but also comprehensible to its intended audience. This underscores the need for initiatives like genomic health education targeting the public, patients, and healthcare professionals. It can take various forms, including educational programs, workshops, clinical interactions, or integration into the informed consent process[39]. Making NGS an optional addition to NBS, contingent upon a clear informed consent, is an option thought to assist parental preferences while maintaining participation in existing NBS programs. This was previously successful with expanded NBS using MS/MS, and it could showcase the health benefits of GS without affecting established public health programs, despite the additional burdens of informed consent and documentation on hospital staff [27, 50].

The flexibility of disease selection is crucial for adaptation to future advancements in technology and understanding[23]. While current focus lies on identifying monogenic diseases, future progress may extend to identifying polygenic disorders and establishing disease risk profiles[37]. Some indicate that, by 2030, up to 1000 genetic disorders may meet criteria for NBS[1].

The NBGS process should involve several steps, including sample collection, accessioning in the laboratory, DNA extraction, quality assessment, sequencing library preparation and genome sequencing. The data is then transferred to a data analysis centre for secondary and tertiary analysis, followed by the transmission of the final report to the physician, who communicates it to the newborn's family[25]. Regarding matters such as the facility site, personnel, equipment and management procedures, compliance with current regulations related to NBS is an option, with a requirement for qualification to perform nucleic acid amplification analysis[17, 35].

Given the medical emergency condition of NB who receive a positive result for certain disorders in NBS, efficient systems for quickly disseminating information between birthing hospitals, public health programs, local providers, and their families are necessary to enable prompt initiation of treatment, particularly for conditions requiring urgent intervention within days[9, 35, 43]. Therefore, creating pre and post-counselling, result interpretation, and follow-up access is

recommended for families where a RD is identified[12,38,43]. The development of regulatory-compliant interpretation software that is continuously updated to match genetic diagnoses with available treatments in childhood is a promising idea for the future[12]. Maintaining partnerships with disease support groups and investing in research facilities, as well as educating and training the broader staff engaged in NBS, are important considerations[9].

Coordination between NGS-based NBS and tNBS programs will be crucial if NGS becomes an adjunct to the current screening process. This will involve aligning the results obtained from both methods for disorders evaluated by both approaches[25]. Challenges associated with revealing VUS or unsolicited findings should be further explored. These may be mitigated through a clear and elucidative gene list and cautious variant curation[3, 38]. Furthermore, establishing a protocol on how to communicate secondary findings and the sharing and storage of data will be essential for managing these issues effectively[38].

Involvement of both the community and stakeholders plays a crucial role in gaining trust and public acceptance. It aids in comprehending public viewpoints regarding ethical dilemmas, for example, determining which information should be disclosed to parents, and it enriches genomic health education initiatives. Community engagement may encompass multiple activities, including active participation in discussions concerning the implementation of WGS or WES, sharing personal experiences, and assisting in the formulation of local and global guidelines[38, 39]. This will create meaningful dialogues, empowering the community to actively participate in decision-making processes within research or screening programs[39].

The widespread embrace of tNBS could be compromised if a new screening test introduces uncertainties regarding its outcomes and advantages. Consequently, its implementation should only proceed when its effectiveness, accuracy, security, and additional value compared to existing programs are thoroughly established, and communities are sufficiently informed about its benefits, risks, and potential difficulties[26].

5. CONCLUSION

NBS is a public health initiative of extreme importance in the lives of many NB, their families and health professionals involved. Looking ahead, NGS holds promise to enhance NBS either as a supplementary test to improve current NBS protocols or as the primary screening method for identifying disorders that lack biochemical markers. In the long run, this approach could reduce the time to diagnoses, enhancing outcomes for those needing prompt diagnosis, improve the success and efficacy of therapies for pediatric patients, and, ultimately, start reducing long-term healthcare expenses. However, NGS cannot yet replace the specificity and quick results provided by conventional MS/MS in detecting many IEM.

Consequently, further investigation on the possibility of implementing a NBGS is still needed. Ultimately, the insights gathered by this review provide useful information to achieve this goal.

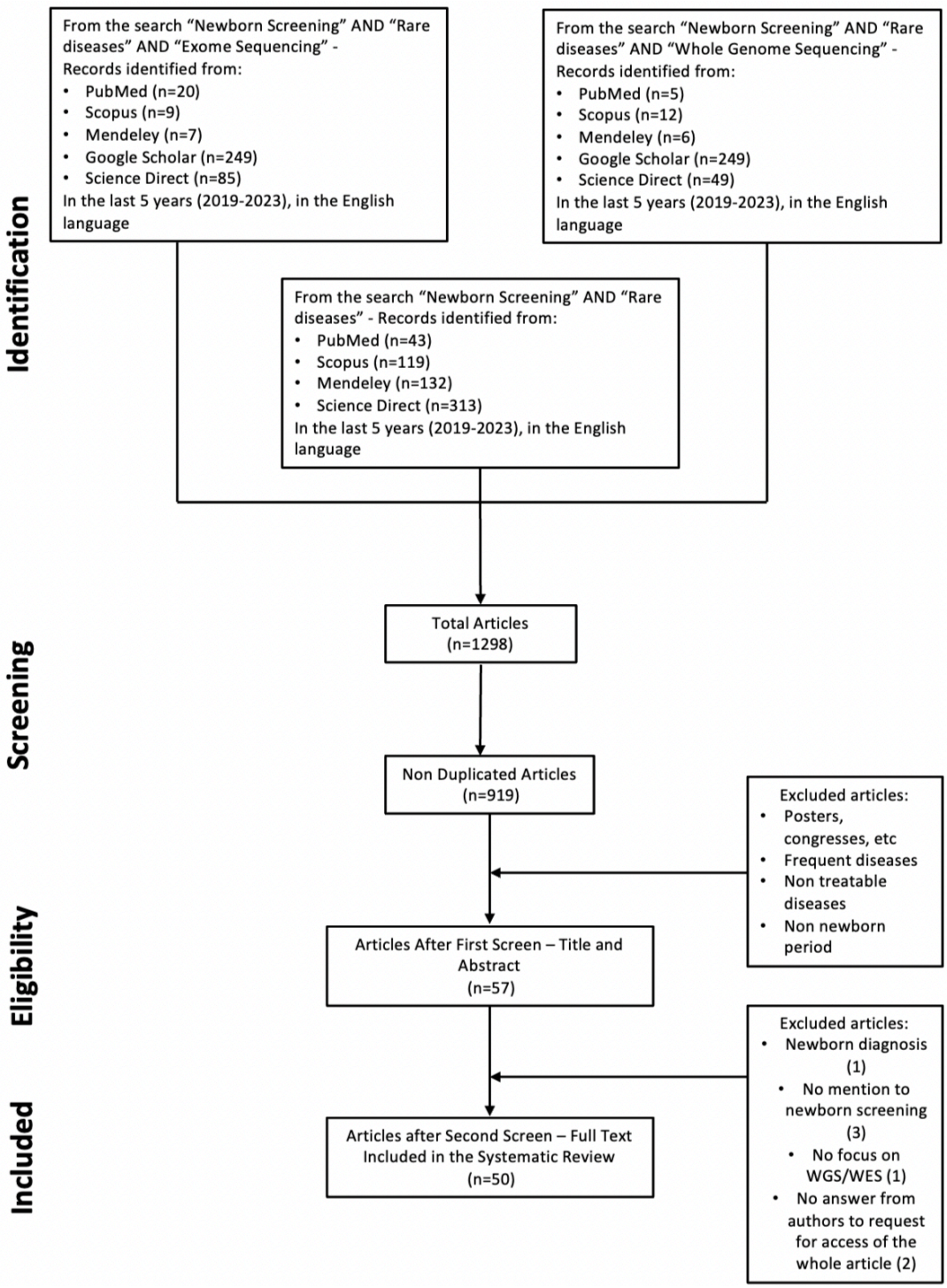

Figure 1 - PRISMA Flowchart of the search, eligibility criteria approaches and study inclusion for the systematic review.

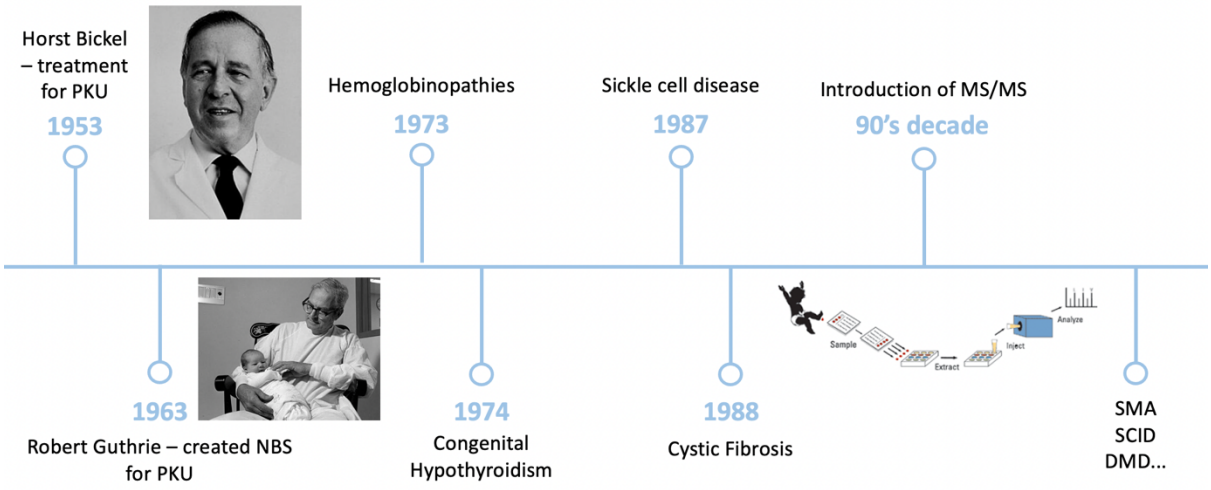

Figure 2 – Newborn Screening Timeline.

6. REFERENCES

1. Kingsmore SF, Smith LD, Kunard CM, *et al.* A genome sequencing system for universal newborn screening, diagnosis, and precision medicine for severe genetic diseases. Am J Hum Genet. 2022;109(9):1605-1619. doi:10.1016/j.ajhg.2022.08.003
2. Brunelli L, Sohn H, Brower A. Newborn sequencing is only part of the solution for better child health. *Lancet Reg Health Am*. 2023;25:100581. doi:10.1016/j.lana.2023.100581
3. Ji C, Farrar MA, Norris S, *et al.* The Australian landscape of newborn screening in the genomics era. Rare Dis Orphan Drug J. 2023; 2(4): 26. doi:10.20517/rdodj.2023.30
4. Cabello JF, Novoa F, Huff HV, Colombo M. Expanded Newborn Screening and Genomic Sequencing in Latin America and the Resulting Social Justice and Ethical Considerations. Int J Neonatal Screen. 2021;7(1):6. doi:10.3390/ijns7010006
5. Clark C, Boardman F. Expanding the notion of "benefit": comparing public, parent, and professional attitudes towards whole genome sequencing in newborns. New Genet Soc. 2022; 41(2), 96–115. doi:10.1080/14636778.2022.2091533
6. Woerner AC, Gallagher RC, Vockley J, Adhikari AN. The Use of Whole Genome and Exome Sequencing for Newborn Screening: Challenges and Opportunities for Population Health. Front Pediatr. 2021; 9:663752. doi:10.3389/fped.2021.663752
7. Huang X, Wu D, Zhu L, *et al.* Application of a next-generation sequencing (NGS) panel in newborn screening efficiently identifies inborn disorders of neonates. Orphanet J Rare Dis. 2022;17(1):66. doi:10.1186/s13023-022-02231-x
8. Kingsmore SF. Dispatches from Biotech beginning BeginNGS: Rapid newborn genome sequencing to end the diagnostic and therapeutic odyssey. Am J Med Genet C Semin Med Genet. 2022; 190(2):243-256. doi:10.1002/ajmg.c.32005
9. Watson MS, Lloyd-Puryear MA, Howell RR. The Progress and Future of US Newborn Screening. Int J Neonatal Screen. 2022 ;8(3):41. doi:10.3390/ijns8030041
10. Garnier N, Berghout J, Zygmunt A, *et al.* Genetic newborn screening and digital technologies: A project protocol based on a dual approach to shorten the rare diseases diagnostic path in Europe. PLoS One. 2023;18(11):e0293503. doi:10.1371/journal.pone.0293503
11. Rudowski S, Boerkoel CF, He M, Kanungo S. Whole Genome Sequencing in Era of Newborn Screening. OBM Genet. 2023; 7(4): 195; doi:10.21926/obm.genet.2304195.
12. Lekstrom-Himes J, Brooks PJ, Koeberl DD, *et al.* Moving away from one disease at a time: Screening, trial design, and regulatory implications of novel platform technologies. Am J Med Genet C Semin Med Genet. 2023; 193(1):30-43. doi:10.1002/ajmg.c.32031
13. Gold NB, Adelson SM, Shah N, *et al.* Perspectives of Rare Disease Experts on Newborn Genome Sequencing. JAMA Netw Open. 2023; 6(5):e2312231. doi:10.1001/jamanetworkopen.2023.12231
14. Horton R, Lucassen A. Ethical issues raised by new genomic technologies: the case study of newborn genome screening. Camb Prism Precis Med. 2022; 1:e2. doi:10.1017/pcm.2022.2
15. Esquerda M, Palau F, Lorenzo D, *et al.* Ethical questions concerning newborn genetic screening. Clin Genet. 2021; 99(1):93-98. doi:10.1111/cge.13828
16. Spiekerkoetter U, Bick D, Scott R, *et al.* Genomic newborn screening: Are we entering a new era of screening?. J Inherit Metab Dis. 2023 ;46(5):778-795. doi:10.1002/jimd.12650
17. Jiang S, Wang H, Gu Y. Genome Sequencing for Newborn Screening - An Effective Approach for Tackling Rare Diseases [published correction appears in JAMA Netw Open. 2024; 7(2):e240325]. JAMA Netw Open. 2023;6(9):e2331141. doi:10.1001/jamanetworkopen.2023.31141
18. Botos L, Szatmári E, Nagy GR. Prenatal and postnatal genetic testing toward personalized care: The non-invasive perinatal testing. Mol Cell Probes. 2023;72:101942. doi:10.1016/j.mcp.2023.101942

19. Shum BOV, Pretorius CJ, Sng LMF, *et al.* Feasibility of Targeted Next-Generation DNA Sequencing for Expanding Population Newborn Screening. Clin Chem. 2023; 69(8):890-900. doi:10.1093/clinchem/hvad066
20. Luo X, Sun Y, Xu F, *et al.* A pilot study of expanded newborn screening for 573 genes related to severe inherited disorders in China: results from 1,127 newborns. Ann Transl Med. 2020; 8(17):1058. doi:10.21037/atm-20-1147
21. Remec ZI, Trebusak Podkrajsek K, Repic Lampret B, *et al.* Next-Generation Sequencing in Newborn Screening: A Review of Current State. Front Genet. 2021;12:662254. doi:10.3389/fgene.2021.662254
22. Wesonga R, Awe O. An Assessment of Traditional and Genomic Screening in Newborns and their Applicability for Africa. Inform Med Unlocked, 2022. 32; 101050
23. Bros-Facer V, Taylor S, Patch C. Next-generation sequencing-based newborn screening initiatives in Europe: an overview. Rare Dis Orphan Drug J. 2023; 2(4): 21. doi:10.20517/rdodj.2023.26
24. Peng G, Shen P, Gandotra N*, et al.* Combining newborn metabolic and DNA analysis for second-tier testing of methylmalonic acidemia. Genet Med. 2019;21(4):896-903. doi:10.1038/s41436-018-0272-5
25. Bick D, Ahmed A, Deen D, *et al.* Newborn Screening by Genomic Sequencing: Opportunities and Challenges. Int J Neonatal Screen. 2022;8(3):40. doi:10.3390/ijns8030040
26. Veldman A, Kiewiet MBG, Heiner-Fokkema MR, *et al.* Towards Next-Generation Sequencing (NGS)-Based Newborn Screening: A Technical Study to Prepare for the Challenges Ahead. International Journal of Neonatal Screening. 2022; 8(1):17. doi:10.3390/ijns8010017
27. Armstrong B, Christensen KD, Genetti CA, *et al.* Parental Attitudes Toward Standard Newborn Screening and Newborn Genomic Sequencing: Findings From the BabySeq Study. Front Genet. 2022;13:867371. doi:10.3389/fgene.2022.867371
28. Bush L, Davidson H, Gelles S, Lea D, Koehly LM. Experiences of Families Caring for Children with Newborn Screening-Related Conditions: Implications for the Expansion of Genomics in Population-Based Neonatal Public Health Programs. Int J Neonatal Screen. 2022;8(2):35. doi:10.3390/ijns8020035
29. Mittal A, Shekhawat D, Joshi V, Singh P, Singh K. Next-generation Sequencing in Newborn Screening: A Review on Clinical and Economic Prospects. The Open Biotechnology Journal. 2023. 17. 10.2174/18740707-v16-e221213-2022-6.
30. Adhikari AN, Gallagher RC, Wang Y, *et al.* The role of exome sequencing in newborn screening for inborn errors of metabolism. Nat Med. 2020;26(9):1392-1397. doi:10.1038/s41591-020-0966-5
31. Wang X, Wang YY, Hong DY, *et al.* Combined genetic screening and traditional biochemical screening to optimize newborn screening systems. Clin Chim Acta. 2022;528:44-51. doi:10.1016/j.cca.2022.01.015
32. Wang X, Guan XW, Wang YY, *et al.* Current attitudes and preconceptions on newborn genetic screening in the Chinese reproductive-aged population. Orphanet J Rare Dis. 2022;17(1):322. doi:10.1186/s13023-022-02474-8
33. van Campen JC, Sollars ESA, Thomas RC, *et al.* Next Generation Sequencing in Newborn Screening in the United Kingdom National Health Service. Int J Neonatal Screen. 2019;5(4):40. doi:10.3390/ijns5040040
34. McBride DJ, Fielding C, Newington T, *et al.* Whole-Genome Sequencing Can Identify Clinically Relevant Variants from a Single Sub-Punch of a Dried Blood Spot Specimen. *Int J Neonatal Screen*. 2023;9(3):52. doi:10.3390/ijns9030052
35. Tong F, Wang J, Xiao R, *et al.* Application of next generation sequencing in the screening of monogenic diseases in China, 2021: a consensus among Chinese newborn screening experts. World J Pediatr. 2022;18(4):235-242. doi:10.1007/s12519-022-00522-8

36. Hao C, Guo R, Hu X, *et al*. Newborn screening with targeted sequencing: a multicenter investigation and a pilot clinical study in China. J Genet Genomics. 2022;49(1):13-19. doi:10.1016/j.jgg.2021.08.008
37. King JR, Grill K, Hammarström L. Genomic-Based Newborn Screening for Inborn Errors of Immunity: Practical and Ethical Considerations. Int J Neonatal Screen. 2023;9(2):22. doi:10.3390/ijns9020022
38. Magnifico G, Artuso I, Benvenuti S. A systematic review of real-world applications of genome sequencing for newborn screening. Rare Dis Orphan Drug J. 2023; 2(3): 16. doi:10.20517/rdodj.2023.17
39. Morley K, Leach B, Hocking L, *et al*. Exploring the ethical dimensions of sequencing newborns' genomes: Rapid literature and evidence review. 2022.
40. Dikow N, Ditzen B, Kölker S, Hoffmann G, Schaaf C. From newborn screening to genomic medicine: challenges and suggestions on how to incorporate genomic newborn screening in public health programs. Med Genet. 2022; 34(1), 13-20. doi:10.1515/medgen-2022-2113
41. La Cognata V, Guarnaccia M, Polizzi A, Ruggieri M, Cavallaro S. Highlights on Genomics Applications for Lysosomal Storage Diseases. Cells. 2020;9(8):1902. doi:10.3390/cells9081902
42. Zou L, Cao Z, He X, *et al.* The targeted exome sequencing strategy (NeoExome) for Chinese newborns with the pilot study of 3423 neonates. Authorea. 2022. 12(1):e2357. doi:10.1002/mgg3.2357
43. Pichini A, Ahmed A, Patch C, *et al.* Developing a National Newborn Genomes Program: An Approach Driven by Ethics, Engagement and Co-design. Front Genet. 2022;13:866168. doi:10.3389/fgene.2022.866168
44. Wu X, Yang Y, Zhou L, Long W, Yu B. Are We Ready for Newborn Genetic Screening? A Cross-Sectional Survey of Healthcare Professionals in Southeast China. Front Pediatr. 2022;10:875229. doi:10.3389/fped.2022.875229
45. Powell SN, Byfield G, Bennetone A, *et al*. Parental Guidance Suggested: Engaging Parents as Partners in Research Studies of Genomic Screening for a Pediatric Population. Front Genet. 2022;13:867030. doi:10.3389/fgene.2022.867030
46. Uveges MK, Holm IA. Current Trends in Genetics and Neonatal Care. Adv Neonatal Care. 2021;21(6):473-481. doi:10.1097/ANC.0000000000000834
47. Chien YH, Hwu WL. The modern face of newborn screening. Pediatr Neonatol. 2023;64 Suppl 1:S22-S29. doi:10.1016/j.pedneo.2022.11.001
48. Wojcik MH, Gold NB. Implications of Genomic Newborn Screening for Infant Mortality. Int J Neonatal Screen. 2023;9(1):12. doi:10.3390/ijns9010012
49. Gold NB, Harrison SM, Rowe JH, *et al.* Low frequency of treatable pediatric disease alleles in gnomAD: An opportunity for future genomic screening of newborns. HGG Adv. 2021;3(1):100059. doi:10.1016/j.xhgg.2021.100059
50. Yamaguchi-Kabata Y, Yasuda J, Uruno A, *et al.* Estimating carrier frequencies of newborn screening disorders using a whole-genome reference panel of 3552 Japanese individuals. Hum Genet. 2019;138(4):389-409. doi:10.1007/s00439-019-01998-7