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Review

Newborn Screening for Neuromuscular Disorders, Disorders of Glycogen Metabolism, and Fatty Acid Oxidation

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Abstract

Newborn screening for neuromuscular disorders and glycogen or fatty acid oxidation disorders aims to identify infants at risk for these conditions, allowing for early intervention and management. While not all neuromuscular disorders currently have established newborn screening programs, there are various disorders for which screening is available or under investigation. Neuromuscular disorders encompass a wide range of conditions that affect the nerve, muscle, or the connection between them. Examples include spinal muscular atrophy (SMA), myotonic dystrophy, and Pompe disease (GSD II). Each disorder has different genetic causes, clinical presentations, and screening approaches. One example of successful newborn screening is for SMA, a genetic disorder caused by the loss of function of the Survival Motor Neuron 1 (SMN1) gene. This screening involves testing newborns' bloodspots for the absence or low levels of SMN1 gene product (protein), and if detected, further confirmatory genetic testing is performed. Early diagnosis of SMA is also crucial for treatments that are now available. In this article, we deal with various types of muscular dystrophy (DMD, BMD, FSHD), mitochondrial diseases, FAO disorders, and carnitine cycle defects.

Keywords

Pompe; SMA; carnitine; fatty acid oxidation disorders; glycogenosis type II.

1. Introduction

Newborn screening for metabolic and neuromuscular disorders is crucial to preventionand care. These screening tests aim to identify infants who may have these disorders early on, allowing for prompt intervention and treatment. Here is the most recent progress in newborn screening for several conditions: neuromuscular disorders, metabolic disorders of glycogen metabolism, and fatty acid oxidation (FAO) disorders that involve abnormalities in the body's chemical processes that affect the breakdown, utilization of glycogen and fatty acids, where newborn screening aims to identify various metabolic disorders, including glycogenosis type II, andFAO disorders and carnitine deficiency.

Newborn Screening (NBS) for metabolic disorders typically involves analyzing a small blood sample taken from the baby's heel within the first few days of life. This blood sample is tested for specific metabolites and enzyme activities that indicate a potential metabolic disorder. If the screening test results are abnormal, further diagnostic testing must be done.

In the field of neuromuscular diseases, progress has been made for spinal muscular atrophies for which NBS is becoming available [1]. Still, NBS for dystrophinopathies, FSHD, LGMD, myotonic dystrophies, and mitochondrial disease therapy is not yet available, and NBS will have to await future developments in treatment [2].

2. Spinal Muscular Atrophies (SMA)

Spinal muscular atrophy (SMA) is a group of genetic disorders with autosomal recessive transmission, characterized by alpha motor neuron degeneration within the spinal cord, leading to muscular weakness and atrophy.

SMA is generally present in families as single cases since it is due to an autosomal recessive genetic defect: both parents of a patient, albeit entirely normal, are carriers [3]. It has been estimated thatabout one SMA child is born every 6-10,000 neonates; healthy carriers are pretty common in the general population, about 2-3%, but specific inbred populations, such as in Arabian countries, carriers and patients have higher frequency. After regulatory approval of the first treatment for SMA, the prospects for care of these patients have changed, including presymptomatic newborn screening and confirmation of diagnosis.

SMA is classified into four main types according to the onset and achieved milestones in type 1 (most severe form, non-sitters), type 2 (sitters), type 3 (walkers that might eventually lose walking ability), and type 4 (walkers). SMN1 is the gene accountable for all SMA types, and more than 90% of SMA cases show the absence of both copies of the gene for deletions and point mutations.

SMA is a group of diseases of two genes: a highly homologous paralog gene, SMN2, is considered a phenotypic modifier, and the number of copies (from 1 to 5) correlates well (but not absolutely) with the phenotypes [3, 4].

NBS for SMA has become increasingly common in many European countries, and there is an effort todetect this disorder early and initiate appropriate interventions.

How many types of SMA?

SMA type 1 is a significant cause of floppy babies with a frog-like posture and represents the most severe form; respiratory distress is frequent in these floppy infants [5], where the advent and results of new treatment have changed natural history and implemented the practical use of NBS.

SMA type 2 (older babies and toddlers) can sit up without help but not stand or walk and have

weak arms or legs and shaking (tremors) in their fingers and hands. Later, they develop joint problems, or scoliosis, requiring ultrasound guidance for delivering through lumbar puncture [6]. Newly available treatments (nusinersen) result in great progress in their life, reachingindependent mobility.

SMA type 3 is less severe than type 2, but it is common. The onset of symptoms is over 18 months, and the course is dependent on several SMN2 copies and presents various phenotypes. The patients can stand and walk without help. However, they may find walking or getting up from a sitting position difficult. SMA type 4 (walkers) might have balance problems, difficulty running or climbing steps, and a slight shaking tremor in their fingers.

These patients have average intelligence and lifelong independence, as these individuals can achievelifestyle independence without treatment.

As for recent progress, three types of drugs are available: Nusinersen (Spinraza) [7], an antisense oligonucleotide; Risdiplam (Evrysdi), a small molecule SMN2 modifier [8]; Onasemnogene abeparvovec-xioi (Zolgensma), an SMN1 gene replacement therapy [9]. The Zolgensma trial showed that all 14 enrolled infants sat independently for ≥30 seconds at any visit ≤18 months. Allsurvived without permanent ventilation at 14 months as per protocol; 13 maintained body weight. No child used nutritional or respiratory support.

Nusinersen treatment at a younger age results in a better motor and respiratory outcome [10, 11], as shown in the phase 2 NURTURE study on presymptomatic infants receiving nusinersen treatment [12]. Overall, nusinersen use in presymptomatic infants seems to have a massive impact on respiratory function. The study conducted by Gonski and colleagues [13] in patients withSMA types 1, 2, and 3 showed respiratory function stability compared to SMA's natural history. Gonski et al. reported 6 patients with SMA type 2 or 3 (age range 2-17 years) who ceased nocturnal invasive ventilation in the two years post-treatment with nusinersen. Risdiplam is a recently approved small molecule for treating SMA, which modifies the splicing of SMN2 pre-messenger RNA, increasing the systemic levels of functional SMN protein. In clinical trials, thepercentages of type 1 SMA patients treated with Risdiplam who met motor milestones and recordedimprovements in motor function were higher than in untreated historical cohorts [14]. Survival without permanent ventilation of Risdiplam-treated patients was 85%, versus 42% of untreated controls [14]. Onasemnogene abeparovec targets type 1 SMA, Nusinersen, and Risdiplam are for pediatric and adult SMA cases.

The meta-analysis assessed the efficacy and safety of Risdiplam in SMA patients in 11 published studies. It showed that these studies demonstrated an improvement in motor function and the effectiveness of respiratory function in SMA type 2 and 3 patients [15, 16]. These encouraging results were based on the availability of NBS in several European countries. There is a call for action in SMA: screening at birth saves lives. National programs are active in Spain, Netherlands, Norway, and Germany, including Italy, where the program is active in Tuscany and Veneto regions. In the US, several States on the East Coast, Midwest, Florida, and California are screening newborns.

3. NBS of Dystrophinopathies

NBS for Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) involves the identification of infants who may be at risk for these conditions shortly after birth. This early detection allows timely intervention and management to improve outcomes for affected individuals. While NBS is established for various situations, its implementation for DMD andBMD is

under investigation.

DMD and BMD are inherited muscle-wasting disorders caused by mutations in the dystrophin geneon the X chromosome. Most dystrophin gene mutations are deletions (65%), and others are point mutations, mainly nonsense and frame-shift mutations. DMD occurs because the mutated DMD gene fails to produce virtually any functional dystrophin [17]. DMD is more severe and generally manifests in early childhood. Large deletions span one or more exons and account for approximately 85% of BMD mutations. BMD presents with milder symptoms and usually appears later in life, with numerous clinical presentations: limb weakness, cardiomyopathy, cramps, and isolated elevated CK [18].

The current gold standard for DMD and BMD diagnosis is genetic testing, which requires a blood or saliva sample for DNA analysis.

Secondary benefits are pertinent to establishing the rationale for NBS in dystrophinopathy cases since it might help reduce the health and economic implications of the disease.

The gold-standard and recommended therapy for DMD patients is based on glucocorticoids [19, 20](prednisone, prednisolone, and deflazacort), which target the glucocorticoid receptor (GR) to exert the anti-inflammation effects by suppressing the pro-inflammatory Nuclear Factor kappa-light- chain-enhancer of activated B cells signaling pathway. However, DMD might present bone loss andosteoporosis, which are exacerbated by glucocorticoid therapy.

In DMD, glucocorticoids are usually administered in daily or intermittent doses after age 4; however, glucocorticoids have different efficacy and remarkable side effects, including

weight gain, osteoporosis, cataracts, hypertension, and stunted bone growth. Bonifati et al. [21] found that the 1220 A to G (Asn363Ser - N363S) polymorphism is essential for a clinical response since patients with this variant in steroid receptors walk longer if treated.

Deflazacort (DF), a glucocorticoid belonging to acetonides or O-isopropylidene derivative, has been used in several DMD series [22, 23]. A clear efficacy picture emerges from the FOR-DMD trial [23],where 196 DMD were randomized, and 164 completed the trial. Both daily prednisone and deflazacort were more effective than intermittent prednisone for the primary outcome. Deflazacort treatment caused less weight gain versus prednisone. Currently, vamorolone, a novel synthetic steroid, has been tried in DMD boys [24, 25] and might be used in mild cases of dystrophinopathy.

Pilot studies have already been completed within the USA, New Zealand, and other countries, suchas Germany. This screening is achieved by measuring CK levels in newborn blood spots and confirmed with genetic testing. The result of NBS in dystrophinopathies before confirmatory genetic testing may also identify patients with LGMDs, as most subtypes are known to have elevated CK. This may be a growing complication of the wide adoption of NBS for DMD/BMD.

4. LGMD's Current Progress and NBS

Limb-girdle muscular dystrophy (LGMD) encompasses a heterogeneous group of genetically diverse disorders that primarily affect the muscles of the hips and shoulders. LGMD can be caused by mutations in several genes, coding for sarcolemmal, nuclear, or other enzymatic proteins. There are different LGMD subtypes with recessive and dominant inheritance patterns [26].

Currently, LGMD is not routinely included in NBS programs for several reasons:

1. Heterogeneity: LGMD comprises multiple subtypes with different genetic causes. Testing for allpossible LGMD genes would be complex and costly. Additionally, different subtypes might

presentwith varying symptoms and ages of onset, making a single screening test difficult to design [26].

2. LGMD has relatively low frequency except in certain regions like the gamma-sarcoglycanopathyform in Tunisia [27].
3. Lack of available therapy: in addition to molecular-based therapies, many non-specific musculardystrophy therapies are currently in clinical trials. Phase 1 studies of AAV-delivered gene therapy for LGMD R3 and R4 (sarcoglycanopathy) have demonstrated proof-of-principle for delivery in anisolated muscle and showed sarcoglycan staining in muscle biopsies post-therapy [28, 29] Preclinical efforts to develop supplementary therapy with ribitol for FKRP mutations (LGMDR9) and gene therapy sarcoglycan-beta mutations (LGMDR4) are underway [30, 31]. NBS in these LGMDs might likely be advisable in the future, but it is not a common practice now.

5. NBS in Glycogenosis Type II

NBS refers to testing newborns for metabolic conditions shortly after birth. Pompe disease, also known as glycogen storage disease type II, is a rare genetic disorder caused by a deficiency of the enzyme alpha-glucosidase (GAA). This enzyme breaks down glycogen stored in lysosomes, particularly in skeletal and cardiac muscles.

NBS for Pompe disease has been implemented in some countries as part of their routine screeningprograms. Screening involves testing for GAA enzyme activity in newborn DBS [32]. There are several reasons why NBS for Pompe disease has been considered and implemented in some countries:

1. Early detection: Pompe disease is a progressive disorder, and early diagnosis allows for early intervention and treatment. Early initiation of enzyme replacement might lead to a reversal of cardiomyopathy and weakness. Different is the status for childhood or affected by late-onset adult forms (LOPD), where symptoms might appear at various ages. Pompe disease myopathological features include vacuolar myopathy, with detrimental autophagosome accumulation resulting in muscle autophagic degeneration [33]. In late-onset GSD II treatment, both FDA and European regulatory agencies approved recently available glucosidase with improved alpha M6P-receptor targeting and enzyme uptake. NeoGAA availability appears to be a step forward in enhancing Enzyme Replacement Therapy (ERT). NeoGAA is available, but its use might change the dosage, targeting tissue delivery. ERT effectively modifies the disease's natural course [34]. Most LOPD cases show an improvement in the first 24 months in a six-minute walk test (6 MWT). Maximal ERT efficacy with al-glucosidase alfa was observed in the first two to three years but declined. Pathophysiologic aspects such as enzyme tissue entry, autophagy, and the response to ERT treatment of motor and respiratory components are considered significant [35, 36]. This new ERT might improve QoL for GSD II patients. The new aval glucosidase alfa with improved M6P-receptortargeting and enzyme uptake is underway [37, 38]. In an extension study, improvements were confirmed in the NeoGAA group and, to a lesser degree, in the group that switched from al-glucosidase alfa to NeoGAA [39].

Another therapeutic strategy, chaperone (50 to 600 mg), resulted in a 2.8-fold increase in GAA activity. In a phase 3 trial, PROPEL combining cipaglucosidase alfa plus miglustat 85 treated LOPD

compared favorably to alglucosidase alpha plus placebo [40], resulting in treatment approval in the EU.ERT might also be improved by combination with other drugs, exercise, and nutrition [41].

Neonatal screening for GSD II is available in several countries, such as Italy [32] and Taiwan. For centers working with NBS, a significant hurdle is the identification of timing for treating LOPD cases.

6. NBS in Mitochondrial Disorders

NBS is underway in mitochondrial diseases for several reasons. While mitochondrial diseases encompass a wide range of conditions, there is ongoing research and consideration regarding their inclusion in NBS. Here are some critical points about NBS in mitochondrial diseases:

1. Importance of early detection: early identification of mitochondrial diseases through NBS is crucial as it allows for prompt intervention and management. Early detection can lead to betteroutcomes and improved quality of life for affected individuals.
2. Challenges in NBS for Mitochondrial Diseases: Implementing newborn screening for mitochondrial diseases poses several challenges. A therapeutic approach to mitochondrial myopathies has consisted of using a cocktail with carnitine, Coenzyme Q/Idebenone, and riboflavin supplement. The treatment of mitochondrial disease depends on the specific symptoms and severity of the condition. Since mitochondrial diseases can affect various organs and systems in the body, a multidisciplinary approach involving different medical specialists is often necessary. Common strategies and treatments used in the management of mitochondrial disease:
    1. Symptomatic treatment: Individual symptoms like seizures, muscle weakness, and gastrointestinal issues can be addressed using standard therapies. For example, antiepileptic medications may be prescribed for seizures, while physical therapy can help improve musclestrength and flexibility.
    2. Nutritional support: Some individuals with mitochondrial disease may benefit from specific dietary modifications such as a ketogenic diet [42] or nutritional supplements and mitochondrial target drugs [43]. This could include a high-caloric diet, coenzyme Q10 supplements, or other vitamins and minerals that support mitochondrial function.
    3. Energy-boosting supplements: supplements such as L-carnitine and idebenone have been triedwith limited success.

Regarding fatigue, a study by van del Loo et al. [44] showed that almost 80% of the patients experienced severe fatigue. This aligns with previous studies, which reported fatigue in 60-100% of the patients [45].

Gene editing is the prospective platform that might, in the future, target mitochondrial DNA as the one developed recently: the ARCUS technology, based on an enzyme found in nature called I-CreI.This editing tool repairs pathological mitochondrial DNA to delete its mutations, as reported at BioWorld [46].

A trial with elamipretide, a new tetrapeptide that targets and stabilizes mitochondrial cardiolipin, wasdone [47]; participants were randomized to receive either 24 weeks of elamipretide 40 mg/day or placebo subcutaneously, but the trial was not successful. Therefore, a selection of cases is applicable.

7. NBS in Myotonic Dystrophy

NBS for myotonic dystrophy (DM) is an active research and development area. Myotonic dystrophy is a genetic disorder characterized by muscle weakness, wasting, and other systemic manifestations. Early detection through NBS can facilitate early intervention and improvepatient outcomes. However, it's important to note that no NBS for myotonic dystrophy has been implemented for now.

Myotonic dystrophy is caused by an expansion of nucleotide repeat sequences, primarily in the DMPK (myotonic dystrophy protein kinase) gene for DM1 [48] or the CNBP (cellular nucleic acid-binding protein) gene for DM2 [49]. The expansion of repeat sequences leads to the production of abnormal RNA molecules that impair cellular function. The severity and age of onset can vary, making early detection and intervention crucial.

The evaluation of NBS strategies for myotonic dystrophy is ongoing. Current research primarily focuses on identifying cases with (CTG)n expansion during prenatal life.

Alleles containing a CTG-repeat length of 51-150 may be asymptomatic or giverise to minimal or classical DM1. A more severe DM1 phenotype is associated with DMPK alleleswith sizes >150 CTG-repeat units.

The core mechanism for DM1 is RNA toxicity, whereby DMPK transcripts with expanded CUG repeats form nuclear condensates that sequester splicing factors in the muscleblind-like (MBNL) family. The resulting loss of MBNL function causes misregulation of alternative splicing and other changes in RNA processing.

DM2 is caused by a tetranucleotide (CCTG)n repeat expansion in the first intron of the CCHC-type zinc finger nucleic acid binding protein (CNPB) gene. The repeat expansion for DM2 is much larger than for DM1, ranging from 75 to over 11000 repeats. Unlike DM1, the size of the repeated DNA expansion does not correlate with age of onset or disease severity.

Mexiletine, an antiarrhythmic drug whose primary mechanism of action is blocking fast sodium channels, has been found effective on myotonia and validated in DM1 and also in nondystrophic myotonia by Statland et al. [50], with few precautions for possible cardiac complications.

In an experimental study in a mouse model, verapamil has been found effective [51]. However, one has to remember the frequent cardiac rhythm involvement in many DM1 cases.

Antisense oligonucleotides (ASOs) have demonstrated promising results in removing CUG-expanded RNA and reversing downstream toxic consequences in both DM1 patient cells and animal models. ASOs target the RNA through base pairing to complementary nucleotide sequences.Much translational research is still needed for NBS and effective therapy for DM1 and DM2.

8. NBS in Facioscapulohumeral Dystrophy

Currently, NBS specifically for Facioscapulohumeral Muscular Dystrophy (FSHD) is not part of routine newborn screening programs but is implemented by some centers.

FSHD is a genetic disorder that affects the muscles, primarily in the face, shoulders, and upper arms. It is an autosomal dominant disease, and the basis of its pathogenesis is ectopic expression of the transcription factor DUX4 in skeletal muscle [52], which leads to the production of the DUX4 protein in muscle cells.

While some countries have implemented NBS for several genetic conditions, FSHD is not currently included in these programs for several reasons:

1. FSHD is relatively rare compared to other genetic disorders that are included in NBS programs. The prevalence of FSHD varies in different populations, but it is estimated to affect approximately 1 in 8,000 to 1 in 20,000 individuals worldwide.
2. Complexity of diagnosis: FSHD diagnosis is not routinely diagnosed in NGS or WES studies or in NGS or WES studies. In addition, the FSHD2 subtype is present. Some techniques address their conduct of complex diagnostic issues [53]. Regarding pathomechanism (s), Gros et al. documented an alteration of IL-6 cytokine [54] both in FSHD cases and animal models.

On this basis, a clinical trial is being conducted to reduce the Il-6 level in FSHD. One has to consider that inflammatory infiltrates in FSHD biopsies are focal and scanty and do not explain the diseasecourse or progression. Another approach is the anti-DUX strategy; the fact that the action of this gene is mostly in early life will need to identify strategies to antagonize FSHD progression. So far,there are no approved disease-modifying therapies for FSHD, an essential consideration for NBS in a patient population, NBS in fatty acid oxidation (FAO) disorders, and carnitine deficiency states.

9. NBS in Fatty Acid Oxidation (FAO) Disorders and Carnitine Deficiency States

NBS for FAO and carnitine cycle disorders is an essential public health initiative aimed at detecting such metabolic disorders in newborn infants. These metabolic diseases affect the body's ability to metabolize fatty acids and utilize carnitine, a molecule transporting fatty acids into the mitochondria for energy production. DBS does the screening process. This blood sample is then tested for specific metabolites that indicate the presence of these disorders. The most commonly tested markers are acylcarnitines, abnormal byproducts of fatty acid metabolism. If the screening test suggests abnormal results, confirmatory testing is performed.

Early detection through NBS allows for early diagnosis and intervention.

Lipid storage myopathy (LSM) is a group of inherited myopathies caused by various gene mutations, which are pathologically characterized by abnormal lipid deposition in muscle fibers.The genetic characteristics of LSM are autosomal recessive inheritance.

Most late-onset LSM patients can be dramatically resolved by riboflavin treatment, so this clinical phenotype is called riboflavin-responsive (RR-LSM or RR-MADD). Riboflavin, also known as vitamin B2, is a coenzyme of some important oxidoreductases in the body and participates in the energy metabolism process in mitochondria. Riboflavin supplements can help with fat metabolism, and earlyrecognition is crucial to improve the prognosis of patients [55].

Regarding carnitine cycle defects, there is a primary carnitine deficiency (PCD) [56] due to OCTN2 gene defect and numerous secondary carnitine deficiency (SCD) states [57]. NBS appears to be an essential tool [58] for disorders of the carnitine cycle.

SCD may result from severe malnutrition, a ketogenic diet, severe malabsorptive states, use of soy milk in preterm infants, and prolonged parenteral nutrition without adequate L-carnitine supplementation [59, 60].

In PCD, plasma carnitine levels are relatively low; hence, it is effective in treating ureaplasma carnitine levels [56]. Patients might also have hepatic or cardiac involvement; they have a moderate degree of skeletal musculardysfunction, cardiomyopathy, and Reye syndrome [61].

Similarly to PCD, hypoketotic hypoglycemia is found in mitochondrial FAO disorders [62], such as those defects due to very long-chain acyl-CoA dehydrogenase, medium-chain acyl-CoA dehydrogenase, or disorders of a defective mitochondrial carnitine-acylcarnitine cycle or disorders

of carnitine shuttle and fatty acid transfer across the mitochondrial membrane, due to defects in criticalenzymes i.e. CPT-II [63, 64] that might manifest with rhabdomyolysis, but do not present LSM. The clinical manifestations of FAO defects and carnitine shuttle disorders are challenging to detect in infancy, although they might usually be triggered by fasting. In PCD, common manifestations include hypoketotic hypoglycemia and cardiomyopathy, and NBS appears effective [54, 55].

In FAO, metabolic abnormalities can first be assessed using the plasma acylcarnitine profile, and metabolic abnormalities can first be evaluated using the plasma acylcarnitine profile of theGC-MS apparatus. Diagnosis is accomplished by molecular testing methods and/or assays of therespective deficient enzyme.

Supplementation with L- L-carnitine is controversial in FAO defects but empirically used. The majority of PCD cases have a good outcome as long as L-carnitine therapy is maintained [65]. Sudden death could be the unexpected manifestation of undiagnosed patients with underlying metabolic defects. In a described case with OCTN2 defect recovery from Reye syndrome [61], a sudden death came after many years of treatment with L-carnitine supplementation. Reye-like syndrome and cardiac abnormalities that include cardiomyopathy might be reversible with carnitine supplementation [66], while if untreated, left ventricular hypertrophy progresses to dilated cardiomyopathy. It causes a decrease in left ventricular ejection fraction and arrhythmias.

Sudden cardiac death has been reported in undiagnosed cases or patients with poor compliance with L- carnitine therapy [67]. Patients should follow a high-carbohydrate and low-fat diet. Alternative energy sources might be provided by the MCT Oil diet, which can enter the mitochondria in the absence of carnitine. Effective treatment of FAO defects and carnitine shuttle disorders involves avoiding fasting, frequent feedings, high carbohydrate, low-fat diets, and carnitine supplementation [67, 68].

NBS for carnitine cycle disorders is performed by blood analysis in the USS and Italy, where NBS identifies most cases of PCD and some FAO defects.

10. Conclusions

NBS in rare metabolic or neuromuscular disorders is challenging due to the heterogeneity of diseasepresentations from multi-systemic organ presentations that impact specific tissues. The panel of diagnostic laboratory exams is different depending on the results of NBS. Some exams require a complex technique, such as the GS- MS apparatus. Still, genetic analysis is also required using conventional Sanger and next-generation techniques.

However, with promising technologies such as gene transfer or gene and RNA editing, personalized and precise medicine appears attainable for a substantial subset of thesedisorders.

This review aims to highlight advances and state-of-the-art aspects of NBS, focusing onvarious metabolic and neuromuscular diseases and exploring the challenges of this highly dynamicfield of research and prevention.

Author Contributions

The author did all the research work of this study.

Competing Interests

The author has declared that no competing interests exist.

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