

Newborn screening and rapid genomic diagnosis of neuromuscular diseases

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Abstract

Background: In recent years, treatments have been approved for certain neuromuscular diseases. In some cases, early pre-symptomatic treatment is necessary for optimal response, and thus newborn screening is critical.

Objective: To review the current status of newborn screening programs for neuromuscular diseases and early diagnosis through genetic testing.

Methods: Following the PRISMA guidelines, a literature search was performed on PubMed for screening of neuromuscular diseases; the search was conducted on literature available as of 1 May 2024.

Results: Included were 77 articles on newborn screening for seven diseases: spinal muscular atrophy (19 studies), Duchenne muscular dystrophy (15), Pompe disease (20), X-linked adrenoleukodystrophy (14), Krabbe disease (6), metachromatic leukodystrophy (2), and myotonic dystrophy 1 (1). Ten articles on rapid genomic diagnosis were identified.

Conclusion: Since 2021, newborn screening programs for neuromuscular diseases have been established, notably in X-linked adrenoleukodystrophy, spinal muscular atrophy, Pompe disease, and Duchenne Muscular Dystrophy. Even in diseases where treatment is currently not life-changing, such as Krabbe disease, new newborn screening programs continue to be implemented, especially in the USA. The use of genetic diagnostic tests does not yet appear to be widespread or at least not widely reported. As new treatments become available, genomic newborn screening programs will need to be rapidly and broadly implemented.

Keywords

neonatal screening, neuromuscular diseases, muscular atrophy, spinal, muscular dystrophy, duchenne, adrenoleukodystrophy, early diagnosis, pompe disease, metachromatic leukodystrophy, myotonic dystrophy 1, krabbe disease

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Introduction

The treatment of patients with neuromuscular diseases (NMD) has evolved considerably in recent years as disease-modifying treatments have been approved for clinical use for patients with several of the approximately 400 described NMDs. The diagnosis of an NMD is frequently delayed due to the rarity and complexity of these diseases.^{1–4} This delay can result in irreversible muscle damage that can greatly reduce the effectiveness of treatment when available.^{5–7} Even in cases where there is no muscle destruction, such as certain forms of congenital myasthenia, the lengthy diagnostic process can lead to decades of compromised quality of life before a correct diagnosis is made and suitable treatment is initiated as well as secondary complications like scoliosis or chest infection.⁸

Improvements of the standard of care and diagnosis workflow, establishment of neuromuscular centers, and increased availability of genomic methods for testing have shortened

the diagnostic odyssey.⁹ Diagnostic delays remain a real issue, however, and are especially harmful for patients who suffer from treatable conditions. Newborn screening (NBS) is a very efficient way of identifying treatable diseases. NBS has been in use for nearly 60 years for various diseases. Incorporation of a test within an NBS program is based on a set of criteria proposed by Wilson and Jungner,¹⁰ with interpretations varying from country to country, particularly as regards

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to the criterion that a treatment be available. In Europe, for example, the approach is conservative with only diseases that are treatable included, whereas in the United States of America (USA) a more flexible approach has been adopted.¹¹

NBS panels previously included only biochemical or metabolic analyses and therefore could not include tests for diseases without metabolic or endocrine markers such as NMDs. Driven by the availability of effective treatment for certain NMDs, genetic NBS is developing very rapidly. A notable example is spinal muscular atrophy (SMA). In just a few years use of a genetic NBS test has become common in high-income countries. The aim of this scoping review is to describe the current status of NBS and early diagnosis of NMD. We carried out a scoping review over the last 10 years to assess the contribution of genetic testing by NBS or whole genome sequencing (WGS) for NMD.

Methods

Literature search

We followed the PRISMA checklist¹² and conducted a thorough literature search using Medline (PubMed). We sought to identify original, full-text articles that discussed NBS tests for NMDs that were published after 1 January 2014 and before 1 May 2024. To identify these articles, we used key terms related to NBS such as ‘neonatal screening’, ‘dried blood’, and ‘Guthrie’, and those related to early diagnosis such as ‘early diagnosis’, ‘whole genome sequencing’ in combination with relevant neuromuscular disease keywords. A detailed search strategy is provided in Supplementary file 1.

Selection of studies

We used the Covidence application to screen the articles. To be included, articles had to be full articles, in English or French, published after 1 January 2014 that had been peer reviewed. Articles had to describe established NBS programs or pilot projects for NMD or describe diagnoses made within the first 28 days of a child’s life. Three reviewers (TD, HL, NB) first screened titles and abstracts for eligibility with the consensus of a minimum of two reviewers required for inclusion. Then two reviewers (TD, HL) reviewed the full text. Reasons for exclusions were recorded. Any potential disagreements were resolved by consensus. When more than one article described the same pilot project, only the most recent was included. We retained studies demonstrating the efficacy of NBS on deidentified Guthrie cards only if there were no pilot projects with identified patients.

Data extraction and presentation

Data were extracted using the Covidence application. Articles were then classified into two categories: NBS or

early diagnosis in symptomatic patients. Articles were then sorted by disease.

Results

Study selection process

The initial searches identified 772 articles describing NBS for neuromuscular disorders. They were screened by title and abstract, and 183 articles were identified for full-text screening. Full-text screening identified 105 articles; 21 articles were added based on reviews of bibliographies or after additional searches of more recent publications. Of these 39 articles were excluded because they described the same studies. In total 77 articles on screening for NMD and 10 on early diagnosis were included in this review. Supplementary file 2 shows the flow chart based on the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines used for the identification of these studies.

Newborn screening programs for NMDs

Spinal muscular atrophy. SMA is a recessive disease caused by homozygous loss-of-function mutations (usually deletions of exon 7) in *SMN1*, the gene encoding a protein critical for maintenance of motor neurons. Three drugs are approved for treatment of SMA: nusinersen,¹³ onasemnogene-abeparvovec,^{14,15} and risdiplam.¹⁶ Extensive data demonstrated that these drugs are most efficacious if treatment begins before symptom onset.^{6,17}

NBS for SMA was reported in 19 articles, which describe 19 studies each conducted in a region or country.^{18–36} The initial implementation of pilot NBS programs for SMA took place in Taiwan in 2014,³⁷ followed by New York in 2016.³⁸ Interestingly, these pilot programs were conducted before any drugs were approved for use in these patients, and some of the patients identified in these pilot programs were included in clinical trials.^{36,39} The incidence rates vary significantly: For example, in Italy, a rate of 1 in 6000 was reported,⁴⁰ whereas in Ontario the reported rate was 1 in 28,000.⁴¹ One of the lowest incidence rates of the disease was found in New York,⁴² which may be due to increased use of preconception screening due to increased communication about the disease.⁴³ In most programs, the first tier of screening is done by quantitative real-time polymerase chain reaction (qPCR), and the second and confirmatory tests are by multiplex ligation-dependent probe amplification (MLPA) or digital droplet PCR. Some programs had issues with false positives, especially at the beginning of the program, and the reasons have been identified and corrected. For example, heparin was used in the tubes used for sampling.²⁸ In recent years, there has been exponential growth in the use of NBS for SMA, and most high-income countries now screen newborns for this disease.⁴⁴ A recent survey showed that 100% of newborns in the USA and 66%

of newborns in Europe now screened for SMA.⁴⁵ Table 1 summarizes the articles that describe NBS for SMA.

Duchenne muscular dystrophy. Duchenne muscular dystrophy (DMD) is the most prevalent childhood form of muscular dystrophy. This condition arises from deletions, duplications, or single nucleotide variants that affect the production of functional dystrophin. First symptoms typically occur when children are 2–3 years old and include proximal weakness and muscle pseudo-hypertrophy. The weakness progresses rapidly and results in the loss of walking between the ages of 7 and 15. On average, there is a two-year gap between the onset of symptoms and diagnosis of DMD.¹ This diagnostic delay not only causes distress for parents, but it can also result in a delay in appropriate care like steroids and multidisciplinary follow-up.

The Food and Drug Administration (FDA) has approved eight drugs for use in patients with DMD: two steroids (deflazacort and vamorolone), four oligo antisense nucleotide that aim to skip exons 51 (eteplirsén, vitolarsén), 53 (golodirsén) and 45 (casimersén), a gene therapy that provides a copy of *microdystrophin* using an AAV-*microdystrophin* construct, and the histone deacetylase inhibitor givinostat. There are age restrictions on some of these drugs. For example, givinostat is for use in patients 6 years and older and the gene therapy is for use in patients 4 years and older. The exon skipping drugs do not have age restrictions. Clinical and pre-clinical efforts toward development of a number of additional potential therapies are ongoing.⁴⁶

Our literature search identified 13 pilot projects or official implementations of NBS for DMD between 1974 and 2022 described in 15 articles.^{47–60} New pilot programs were recently launched in the USA; the main objectives are to evaluate the feasibility and benefit of NBS for DMD.^{51,61} In the USA, there is considerable pressure from parent associations to add DMD to the list of diseases in the Recommended Uniform Screening Panel (RUSP), even though evidence for efficacies of available treatments is limited. One of the rationales of this position is to avoid recurrence of the disease in the family. In screening efforts reported to date, the incidence in young males is between 1 in 3500⁴⁸ to 1 in 10,300.⁴⁹ Table 2 summarizes the articles that describe NBS for DMD.

X-linked adrenoleukodystrophy. X-linked adrenoleukodystrophy (X-ALD) is a peroxisomal impairment caused by a loss of function mutation in the *ABCD1* gene located on the X chromosome. This metabolic disorder affects the adrenal glands, brain, and spinal cord, resulting in devastating consequences. Boys who are hemizygous are more severely affected by X-ALD than are heterozygous girls (60% of cases). If left untreated, subjects with X-ALD typically survive only a few years after symptom onset. Treatments include corticosteroids for adrenal insufficiency, hematopoietic stem cell transplantation, and gene therapy. Treatment during the initial stages of brain inflammation prior to

neurologically debilitating brain adrenoleukodystrophy enhances survival rates and improves functional outcomes.⁶²

NBS for X-ALD was reported in 14 articles that describe 13 studies, each conducted in a different region or country,^{63–67} most of them in the USA.^{62,68–75} Most of these articles were published within the last five years, a sign of the rapid development of NBS programs of X-ALD. In the USA, the program, which was first implemented in 2016, has proven to be effective. The first-tier tests for X-ALD are all metabolic, but confirmatory tests are increasingly genetic. The incidence of X-ALD in males varies from 1 in 4000⁷⁶ to 1 in 14,000.⁶⁹ Table 3 summarizes the articles that describe NBS for X-ALD.

Pompe disease. Pompe disease, also called glycogenosis type 2, is an autosomal recessive lysosomal storage disorder resulting from acid alpha-glucosidase (GAA) deficiency. The disease has a broad clinical spectrum, ranging from the infantile form, which manifests within the first few months of life, to adult forms. Without treatment, the infantile form invariably results in early mortality due to cardiorespiratory failure or respiratory infection, typically before the age of one year. In the later-onset forms, symptoms can emerge at any age and are linked to progressive skeletal muscle dysfunction.

Enzyme replacement therapy (ERT) with alglucosidase alfa (Myozyme) received approval from the FDA and the European Medicines Agency (EMA) in 2006.⁷⁸ In 2023, the FDA approved the combination therapy of cipaglucosidase alfa-atga with miglustat to treat adults who are not improving on their current regime of ERT.⁷⁹ Early intervention with ERT is associated with improved outcomes.⁸⁰ Gene therapy development is mostly focused on restoring GAA production and is currently in clinical development.

NBS for Pompe disease was reported in 20 articles describing 18 studies. The inaugural NBS pilot program was launched in Taiwan in 2005 and has been consistently documented since.^{81,82} Since 2015, Pompe disease has been included in the RUSP in the USA and screening for Pompe disease is part of NBS programs in 38 states.⁸³ These efforts have been described in a number of publications.^{84–92} Comparable initiatives have been established in Italy,⁹³ Mexico,⁹⁴ Japan,^{95,96} China,⁹⁷ and Brazil.^{98–100} The reported incidences for infantile and late forms vary from 1:10,600 in Brazil to 1:38,000 in Japan. Table 4 summarizes the articles that describe NBS for Pompe disease.

Krabbe disease. Krabbe disease is an autosomal recessive lysosomal disorder that impacts the white matter of both the central and peripheral nervous systems. The severity depends on the age of onset. This disease is caused by loss-of-function mutations in both alleles of the *GALC* gene, leading to a deficiency in galactosylceramidase. Historically, 85–90% of patients are diagnosed with the infantile form, the most severe type, which manifests within the first six years of life. In cases where the disease begins within the

Table 1. NBS for SMA.

Country (region)	Date	Pop	Total number	Scr First-tier	Scr 2nd-tier	Conf	Cons	First result	N° cases	Prev	Aim of study
Taiwan ¹⁸	2014-2019	NB	364,000	qPCR	ddPCR	MLPA	opt-in	21	21	1/17,000	Explore SMA NBS and treatment of identified subjects
USA (NY) ¹⁹	2016-2017	NB	3826	qPCR		qPCR	opt-in	1	1	1/3826	To determine feasibility of SMA NBS
Germany ²⁰	2018-2020	NB	297,163	qPCR		MLPA	opt-in	43	43	1/6910	Report on 2-year pilot of SMA NBS
Belgium (FWB) ³⁶	2018-2021	NB	136,339	qPCR	qPCR MLPA	MLPA	opt-out	9	9	1/13,364	Explain start of SMA NBS
Australia (NSW/ACT) ²¹	2018-2021	NB	252,081	qPCR	ddPCR	ddPCR	opt-out	22	21	1/11,458	Evaluate implementation of SMA NBS
USA (NC) ²²	2018-2020	NB	12,065	qPCR	qPCR	qPCR	opt-in	2	1	1/12,065	Evaluate SMA NBS
Italy (Tuscany, Lazio) ²³	2019-2021	NB	90,885	qPCR	qPCR	qPCR	opt-in	15	15	1/6059	Evaluate SMA NBS for extension
USA (GA) ²⁹	2019-2020	NB	301,418	qPCR	N/A	N/A	opt-out	39	15	1/18,840	Results of SMA NBS program: Incidence, timing
USA (WI) ³¹	2019-2020	NB	60,984	qPCR	ddPCR	ddPCR	opt-out	6	6	1/10,164	Report on 1-year SMA NBS program
Canada (Ontario) ²⁶	2020-2021	NB	139,810	Mass Array	MLPA	MLPA	opt-out	5	5	1/27,960	Report on beginning of SMA NBS
Japan (Osaka) ²⁴	2020-2023	NB	105,419	qPCR	MLPA	MLPA	opt-in	1	1	1/18,494	Evaluate SMA NBS
USA (CA) ²⁵	2020-2021	NB	628,791	qPCR	ddPCR	PCR	opt-out	34	34	1/18,494	Report on beginning of SMA NBS
Japan (Hyogo) ²⁸	2021-2023	NB	16,037	qPCR		MLPA qPCR	opt-in	17	3	1/5346	Report on 2.5-year program of SMA NBS
Japan (Kumamoto) ³⁰	2021-2022	NB	13,587	qPCR	qPCR	MLPA	opt-in	1	1	1/13,587	Report on SMA NBS program
Russia (8 regions) ³²	2022	NB	202,908	qPCR	qPCR	MLPA	opt-in	32	26	1/7804	Report on SMA NBS program
Russia (St Petersburg) ³³	2022	NB	36,140	Genome X real-time	PCR		opt-in	4	4	1/9035	Report on SMA NBS program
Ukraine ²⁷	2022-2023	NB	65,880	PCR-based							
Croatia ³⁴	2023	NB	32,655	qPCR	qPCR	MLPA	opt-in	11	11	1/5989	Report on implementation of SMA NBS program
Serbia ³⁵	2023	NB	54,393	qPCR	MLPA	MLPA	opt-out	5	5	1/6531	Report on SMA NBS program
							opt-out	2	2	1/9066	Report on implementation of SMA NBS program and results of the national program.
All			2,759,988					268	222	1/12,432	

List of abbreviations: Conf: Confirmatory assay; Cons: parents' method of consent; ddPCR: digital droplet PCR; First result: first result positive or inconclusive; FWB: Federation Wallonia-Brussels (Region of Belgium); CA: California; GA: Georgia; MLPA: multiplex ligation-dependent probe amplification; NB: Newborn; N° cases: Number of confirmed cases; N/A: not available; NC: North Carolina; NSW/ACT: New South Wales and Australian Capital Territory (region of Australia); NY: New-York; Pop: description of the population screened; Prev: Prevalence; qPCR: quantitative real-time polymerase chain reaction; Ref: references used; Scr: Screening assay; WI: Wisconsin.

Table 2. NBS for DMD.

Country	Date	Pop	Total number	Scr First-tier	Scr 2nd-tier	Conf	Cons	First result	N° cases	Prev	Aim of study
Germany ^{47,48}	1974–2011	NB (M)	537,000	CK assay	/		Opt-in		155	1/3500	Evaluation of DMD NBS
France ⁵³	1975–1986	NB (M)	218,851	CK assay	/	Clinical assessment	N/A		48	1/4600	Report on DMD NBS program
New Zealand ⁵²	1979	NB (M)	10,000	CK assay	/	Clinical assessment	N/A		2	1/5000	Report on DMD NBS program
Canada (Manitoba) ^{47,57}	1986–2007	NB (M)	172,860	CK assay	/	DMD molecular testing	Opt-out		18	1/9600	Determine whether screening reduces number of second children born with DMD. Observation of development.
USA (PA) ⁴⁹	1987–1995	NB (M)	403,576	CK assay	CK isozyme	DMD molecular testing / muscle biopsy	Opt-out, verbal consent		39	1/10,300	Description of attitudes of parents and of patients with diagnosis was with DMD NBS or not.
UK (Wales) ⁵⁰	1990–2011	NB (M)	369,780	CK assay	/	DMD molecular testing / plasma CK	Opt-in	145	56	1/6600	Report on 21-year DMD NBS pilot program.
Cyprus ⁵⁶	1992–1997	NB (M)	30,014	CK assay	/	DMD molecular testing / muscle biopsy	Opt-out	43	5	1/6000	Evaluation of the methods and implementation of DMD NBS pilot program.
USA (OH) ⁵⁵	2007–2010	NB (M)	17,865	CK assay	/	DMD molecular testing	Opt-in	168	3	1/6000	Evaluate of methods and feasibility of DMD NBS.
China (Zhejiang) ⁵⁴	2017	NB (M)	18,424	CK-MM assay	/	DMD molecular testing	Opt-in	13	4	1/4600	Recommendations for follow-up care.
China (Henan) ⁵⁹	2019–2019	NB (M)	13,110	CK-MM	MLPA	NGS and sanger sequencing	Opt-in	3	3	1/4370	Determine optimal cutoff value and evaluate assess detection rate.
USA (NY) ⁵¹	2019–2021	NB	36,781 (M: 18,654)	CK-MM	CK-MM	NGS	Opt-in	42	3	M: 1/6218	Evaluate the feasibility and benefit of DMD NBS.
USA (NC) ⁵⁸	2020–2022	NB	13,354 (M: 6759)	CK-MM	Total CK	NGS	Opt-in	84	2	M: 1/3380	Implement and evaluate DMD NBS.
Taiwan ⁶⁰	2021–2021	NB	50,572 (M: 26,130)	CK-MM	CK-MM	WES	Opt-out	632	3	M: 1/8710	Establish that DMD NBS enables earlier management
All			M: 1,843,023					1392	341	M: 1/5404	

List of abbreviations: CK-MM: creatine kinase muscle; First result: first result positive or inconclusive; Conf: Confirmatory assay; Cons: parents' method of consent; First result: Positive first result or inconclusive M: male; N/A: not available; NGS: next-generation sequencing; NY: New York; NB: newborn; N° cases: Number of confirmed cases; NGS: Next-generation sequencing; OH: Ohio; PA: Pennsylvania; Pop: description of the population screened; Prev: Prevalence; Ref: references used; Scr: Screening assay; WES: whole exome sequencing.

Table 3. NBS for X-ALD.

Country	Date	Pop	Total number	Scr First-tier	Scr 2nd-tier	Conf	Cons	Result	N° cases	Prev	Aim of study
USA (NY) ⁷⁵	2014–2015	NB	365,000	FIA-MS-MS		Sanger sequencing	N/A	33	M: 13 F: 13 (4 Zellweger Syndrome)	All: 1/28,076	Report advisory committee recommendation.
Taiwan ⁶³	2016	NB	320,528	FIA-MS-MS	HPLC-MS-MS	WGS	Opt-in	43	M: 12	M: 1/13,825	Evaluate extended national NBS program.
USA (CA) ⁶⁹	2016–2020	NB	1,854,631	FIA-MS-MS	LC-MS-MS	Sanger sequencing	Opt-out	355	M: 95 F: 110 (23 Zellweger Syndrome)	M: 1/14,397	Describe NBS experience.
USA (MN) ⁷⁶	2017–2018	NB	67,836	FIA-MS-MS	/	Molecular testing + Very-Long Chain Fatty Acids analysis	Opt-out	56	M: 9 F: 5	All: 1/4845 Male: 1/3878	Report on NBS.
USA (GA) ⁷⁰	2017	NB	51,081	FIA-MS-MS	LC-MS-MS	Gene sequencing	Opt-out	11 (M: 4, F: 7)	M: 1 F: 3	All: 1/51,081	Report on use of two-tiered strategy of NBS for ALD
USA (PA) ⁷¹	2017–2021	NB	542,554 (M: 278,330)	FIA-MS-MS	LC-MS-MS	NGS of the ABCD1 gene	Opt-out	51	M: 21 F: 23	M: 1/13,110	Review data and outcomes of NBS program
USA (NC) ^{72,77}	2018	NB	52,301	HPLC-MS-MS		Sanger sequencing	Opt-out	12	3	1/10,000	Describe NBS pilot
USA (NE) ⁷³	2018–2021	NB	82,920 (M: 42,332)	FIA-MS-MS	HPLC-MS-MS	Gene-specific long-range PCR for genomic sequencing	Opt-out	23 (M: 9 F: 14)	M: 4 F: 9	M: 1/10,583	Summarize initial experience with NBS.
USA (IL) ⁷⁴	2019–2021	NB	276,000	LC-MS-MS	Measurement of plasma VLCFA	Gene sequencing	Opt-out	18 (M: 7, F: 11)	M: 4 F: 8	1/16,200	Report outcomes of initial NBS program.
China ⁶⁴	2020–2021	NB	43,653	LC-MS-MS		NGS	Opt-in	32 (M: 18 F: 14)	M: 7 F: 7 (2 other diseases)	1/6236	Evaluate performance NBS program.
Italy ⁶⁵	2021–2024	NB	started	FIA-MS-MS	UHPCLC-MS-MS	NGS	Opt-in	/	/	/	Improve diagnosis and subsequent treatment.
Japan ⁶⁶	2021–2025	NB	1000	HPLC-MS-MS	HPLC-MS-MS	VLCFA and ABCD1 genetic analyses	Opt-in	0	0	/	Improve prognosis of patients.
The Netherlands ⁶⁷	2021	NB (M)	71,208	FIA-MS-MS	HPLC-MS-MS	Gene sequencing	Opt-in	507 (M: 249)	4	All: 1/18,000 M: 1/9000	Report on boys-only pilot study of NBS.
All			All: 3,728,712					M: 837	M: 169	All: 1/22,063	

List of abbreviations: CA: California; Conf: confirmatory assay; Cons: parents' method of consent; F: female; FIA-MS-MS: flow injection analysis tandem mass spectrometry; First result: first result positive or inconclusive; GA: Georgia; HPLC: high-performance liquid chromatography; IL: Illinois; LC-MS-MS: liquid chromatography coupled to tandem mass spectrometry; M: male; MN: Minnesota; MS-MS: tandem mass spectrometry; N° cases: Number of confirmed cases; NB: newborn; NC: North Carolina; NE: Nebraska; NGS: next-generation sequencing; PA: Pennsylvania; Pop: description of the population screened; Prev: prevalence; Ref: references used; Scr: screening assay; UHPCLC: ultra-high performance liquid chromatography; VLCFA: very long chain fatty acid.

Table 4. NBS for pompe disease.

Country	Date	Total Pop number	Scr First-tier	Scr 2nd-tier	Conf	Cons	First result	N° cases	Prev	Aim of study
Taiwan ⁸¹	2005– 2018	NB 994,975	GAA activity	/	lymphocyte GAA activity / GAA WGS	Opt-in	55	16 IOPD 39 LOPD	IOPD: 1/62,186 LOPD: 1/25,512 Ali: 1/ 18,090	Demonstrate advantage of early diagnosis and treatment, even for LOPD.
Italy ⁹³	2015– 2018	NB 112,446	FIA-MS-MS	LC-MS/MS	Enz + Mol test + Urinary GAGs analysis + CK	Opt-in	28	2 IOPD 6 LOPD	IOPD: 1/56,223 LOPD: 1/18,741 Ali: 1/ 14,055	Report on NBS regional program.
Taiwan ⁸²	2010– 2021	NB 1,228,539	GAA activity		GAA gene sequencing	Opt-in	33	33 IOPD	IOPD: 1/37,228	Demonstrate diagnostic and treatment strategies for IOPD.
Mexico ⁹⁴	2012– 2016	NB 20,018	GAA activity	/	Enz + Mol test	Opt-in	19	1	Ali: 1/20,000	Evaluate the results of a lysosomal NBS.
USA (WA) ⁸⁴	N/A	NB 110,000	GAA activity MS/ MS		GAA gene sequencing	N/A	13	4	Ali: 1/27,800	To assess the performance of MS/MS.
USA (NYS) ⁸⁵	2013– 2014	NB 19,197	LC-MS-MS			Opt-in	6	0 IOPD 1 LOPD	1/19,197	Report on pilot NBS program.
USA (MO) ¹⁰¹	2013– 2018	NB 467,000	Fluor Digital Micro-fluidics	/	Enz + Mol test + Urinary GAGs analysis + CK	Opt-out	274	10 IOPD 36 LOPD	IOPD: 1/46,700 LOPD: 1/13,000 Ali: 1/ 10,200	Report on 6-year NBS program.
Japan ^{95,96}	2013– 2020	NB 296,759	Fluor assay	/	NGS	Opt-in	154	1 IOPD 7 LOPD	IOPD: 1/296,759 LOPD: 1/42,394 Ali: 1/38,094	Summarize results of NBS program.
USA (IL) ¹⁰²	2015– 2019	NB 684,290	LC-MS-MS	2-tiered cut-off system	Enz + Mol test + Urinary analysis	Opt-out	397	3 IOPD 26 LOPD	IOPD: 1/228,100 LOPD: 1/26,300 Ali: 1/23,600	Description of NBS program.
USA (PA) ⁸⁷	2016– 2019	NB 531,139	FIA-MS-MS	/	Enz + Mol test	Opt-out	115	2 IOPD 31 LOPD	IOPD: 1/265,500 LOPD: 1/17,100 Ali: 1/16,100	Evaluation of benefits and challenges of NBS.
Brazil (Porto Alegre) ^{98,99}	2016–N/ A	NB 10,527	DMF		NGS	Opt-out	N/A	0 IOPD 1 LOPD	Ali: 1/10,527	Evaluation of challenges of NBS.
USA (KY) ⁸⁸	2016– 2017	NB 55,161	FIA-MS-MS	CLIR tools		Opt-out	15	2	LOPD: 1/27,581 Ali: 27,581	Report on pilot NBS program.
USA (WI) ⁹²	2017– 2019	NB 99,018	N/A	N/A	N/A	N/A	12	0 IOPD 12 LOPD	Ali: 1/8251	Report on pilot NBS program.
USA (MN) ⁸⁹	2017– 2021	NB 257,726	FIA-MS-MS	FIA-MS-MS	GAA sequencing	Opt-out	21	2 IOPD 15 LOPD	OIPD: 1/128,863 LOPD: 1/17,182 Ali: 1/15,160	Evaluate diagnostic and follow-up outcomes.
USA (CA) ⁹⁰	2018– 2019	NB 453,152	FIA-MS-MS	Mol test	Mol test	Opt-out	88	2 IOPD 16 LOPD	IOPD: 1/226,600 LOPD: 1/28,300 Ali: 1/25,200	Report on 1-year NBS program.
Brazil ¹⁰⁰	2018–N/ A	NB 20,066	FIA-MS-MS		NGS	Opt-out	8	0 IOPD 5 LOPD	Ali: 1/10,600	Evaluation of challenges of NBS.
USA (GA) ⁹¹	N/A	NB 59,332	FIA-MS-MS	FIA-MS-MS		Opt-out	5	1 IOPD 2 LOPD	IOPD: 1/59,332 LOPD: 1/29,666 Ali: 1/ 19,777	Evaluate implementation of NBS.

(continued)

Table 4. Continued.

Coun-try Aim of study	Date	Pop	Total number	Scr First-tier	Scr 2nd-tier	Conf	Cons	First result	N° cases	Prev
China (Shangai) ⁹⁷	2021	NB 50,108	FIA- MS-MS	Pompe	NGS	Opt-in	10	3 LOPD	LOPD: 1/16,702	Evaluate the birth prevalence of and determine subclinical forms.
All		5,469,453					1254	LOPD: 72 LOPD: 200 All: 286	LOPD: 1/75,964 LOPD: 1/27,347 ALL: 1/19,123	

List of abbreviations: Conf: Confirmatory assay; Cons: parents' method of consent; DMF: digital micro-fluidics; Enz: Enzymatic testing; FIA-MS-MS: flow injection analysis tandem mass spectrometry; First result: first result positive or inconclusive; GA: Georgia; GAA: acid α -glucosidase; IOPD: infantile-onset Pompe disease; LC-MS-MS: liquid chromatography coupled to tandem mass spectrometry; LOPD: late-onset Pompe disease; Mol Test: molecular testing; MS-MS: tandem mass spectrometry; N/A: not available; NB: newborn; NGS: next-generation sequencing; Pop: description of the population screened; Prev: Prevalence; Ref: references used; Scr: Screening assay; Urinary GAGs analysis.

first year, rapid neurological decline is observed, often resulting in death before the age of two. Late-onset Krabbe disease presents with much more variability in symptoms and progression.¹⁰³ The rarity of the disease poses a challenge for evaluating the effectiveness of pre-symptomatic treatments. Krabbe disease has proven resistant to most single-therapy treatments. Hematopoietic stem cell transplantation, the current standard of care, can modify the disease's progression, but it only slows it down, even if begun pre-symptotically. Recent successes with combination therapies in small animal models and the discovery of new pathogenic mechanisms offer hope for developing effective treatments for this devastating condition.^{104,105}

Six studies have evaluated NBS for Krabbe disease: three in the USA,^{88,106,107} one in Mexico,⁹⁴ one in China,⁹⁷ and one in Brazil.¹⁰⁰ Given the low incidence of the disease, estimated at 1 in 100,000,¹⁰⁵ not all programs have identified cases. Attempts to include Krabbe on the RUSP have been twice unsuccessful and in January of 2024, the Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) officially recommended that Krabbe disease be added to the RUSP. It is currently implemented in only eleven states in the USA. Recent publications have raised ethical concerns regarding such screening,¹⁰⁸ even though patient associations are pushing for its implementation.¹⁰⁹ Table 5 summarizes the articles that describe NBS for Krabbe disease.

Metachromatic leukodystrophy. Metachromatic leukodystrophy (MLD) is a metabolic disorder inherited in an autosomal recessive manner, characterized by the accumulation of sulfatides in the central and peripheral nervous systems due to a deficiency in the enzyme arylsulfatase A, resulting in demyelination. The disease primarily manifests as a decline in motor or cognitive functions. Depending on the subtype, the disease has a highly variable progression and duration but eventually leads to severe disability and death. Both the EMA and the FDA have approved atidarsagene autotemcel, an autologous hematopoietic stem cell gene therapy, for the treatment of children diagnosed with pre-symptomatic late infantile, pre-symptomatic early juvenile, or early symptomatic early juvenile MLD.

The first pilot project aimed at evaluating the feasibility of NBS for MLD was conducted in the USA in 2020, screening 27,335 de-identified dried blood spots.¹¹⁰ Very recently, a study in Manchester, UK showed the effectiveness of a sulphate test on dried blood spots and the evaluation of ARSA enzyme activity as a two-tier strategy.¹¹¹ One infant was identified. This program could soon be recommended by the UK National Screening Committee for incorporation into routine NBS. Table 5 summarizes the articles that describe NBS programs for MLD.

Myotonic dystrophy 1. Myotonic dystrophy 1 (MD1) is an autosomal dominant disorder marked by muscle weakness, myotonia, early onset cataracts, and various systemic

Table 5. NBS for Krabbe disease, MLD, and MDI.

Disease	Coun-try	Date	Pop number	Total	Scr First-tier	Scr 2nd-tier	Conf	Cons	First result	N° cases	Prev	Aim of study
Krabbe	USA (NY) ¹⁰⁶	2006–2014	NB 2,090,910	LC-MS-MS	Mol test		Enz	Opt-out	620	5	1/418,000	Report on NBS program.
	Mexico ⁹⁴	2012–2016	NB 20,018	LC-MS-MS	/		Enz and Mol Test	Opt-in	38	0		Evaluation of results of lysosomal NBS.
	USA (KY) ⁸⁸	2016–2017	NB 55,161	FIA-MS-MS	CLIR tools			Opt-out	181	1	1/55,000	Report on NBS program.
	USA (IL) ¹⁰⁷	2017–2020	NB 494,147	LC-MS-MS	Mol test + psychosine levels		Follow-up	Opt-out	288	2 IOKD 6 LOKD	IOKD: 1/250,000 LOKD: 1/82,400 Ali: 1/61,800	Report on NBS program and role of psychosine in disease diagnosis.
MLD	Brazil ¹⁰⁰	2018-N/A	NB 20,066	MS-MS		UHPLC MS-MS	NGS	Opt-out	1	0		Evaluation of challenges of NBS.
	China (Shanghai) ⁹⁷	2021	NB 50,108	FIA-MS-MS (GALC)	Krabbe		NGS	Opt-in	20	9 LOKD	LOKD: 1/5567	Evaluation of birth prevalence and prevalence of subclinical forms.
	USA (WA) ¹¹⁰	2023-N/A	DBS 27,335	LC-MS-MS	Enz		Mol test	no	195	2		Assessment of feasibility of NBS.
	UK (Manchester) ¹¹¹		DBS 3687	Sulphatides enz test - UPLC-MS-MS	PCR: ARSA gene sequencing		Enz sulphamidase and β-galactosidase	no	11	1	1/3687	Assessment of feasibility of NBS.
MDI	USA (NY) ¹¹²	2013–2014	DBS 51,341	triplet primed-PCR + melt curve analysis			Mol test	no	143	24	1/2100	Determination of prevalence.

List of abbreviations: Conf: Confirmatory assay; Cons: parents' method of consent; DBS: deidentified; Enz test: enzymatic testing; FIA-MS-MS: flow injection analysis tandem mass spectrometry; First result: first result positive or inconclusive; IOKD: infantile-onset Krabbe disease; LC-MS-MS: liquid chromatography coupled to tandem mass spectrometry; LOKD: late-onset Krabbe disease; Mol Test: molecular testing; MS-MS: tandem mass spectrometry; N° cases: Number of confirmed cases; N/A: not available; NB: newborn; NGS: next-generation sequencing; Pop: description of the population screened; Prev: Prevalence; Ref: references used; Scr: Screening assay; UHPCLC MS-MS: ultra-high-performance liquid chromatography MS-MS; UPLC MS-MS: ultra-performance liquid chromatography MS-MS.

Table 6. Rapid genomic diagnosis of NMD.

Country	Date	Pop	Total number	Scr First-tier	N° cases	Aim of study
China ¹¹⁷	2012–2014	< 28 days	186 NB with hypotonia	- Chromosome karyotyping - LC-MS-MS - LS-PCR - MLPA - Sanger sequencing - NGS	117 (22 peripheral hypotonia disorders)	Evaluate speed and diagnostic specificity
USA ¹¹⁴	2015–2017	<28 days	20 acutely ill infants	Targeted gene panel sequencing	10 (1 congenital presynaptic myasthenic syndrome 6)	Evaluate speed and cost-effectiveness of the diagnosis
India ¹¹⁶	N/A	Fetus /NB	4 GSD IV (with severe neuromuscular form)	WES	4 severe perinatally lethal neuromuscular forms of GSD IV	Find novel pathogenic variants
Sweden ¹²⁰	N/A	NB	1 hypotonic NB with respiratory distress	WES	Myopathy in Shwachman–Diamond syndrome	Diagnosis
USA ¹¹³	2021 –N/A	<28 days	1 NB with hypotonia, dysmorphic features, dysphagia	WGS	Neonatal-onset MYH2 hereditary myosin myopathies	Diagnosis
China ¹¹⁸	2019–2020	NB	93 NB small for gestational age	- TMS - Angel Care genomic screening (panel of 150 diseases) - tNGS	45 (4 Krabbe disease, 3 cerebrotendinous xanthomatosis, 2 SMA, 1 primary carnitine deficiency)	Examine the clinical application of genomic screening in newborns small for gestational age
UEA ¹²¹	2021–2022	1–90 days	5 critically ill children	Rapid WGS	3 (1 dystrophy–dystroglycanopathy)	Diagnosis
Belgium ¹²²	2021–2022	2 days–18 years	21 critically ill children	Rapid WGS	12	To establish the path for a nationwide semi-centered rapid WGS network in Belgium.
India ¹¹⁵	N/A	NB	1 floppy NB	Clinical exome sequencing	Congenital hypomyelinating neuropathy	Diagnosis
South Korea ¹¹⁹	N/A	NB	111 (55 NB - hypotonia - seizures - skeletal dysplasia)	tNGS	15 (1 congenital myasthenic syndrome)	Evaluate the possibility of rapid and timely diagnosis of treatable rare genetic diseases

List of abbreviations: LC-MS-MS: liquid chromatography coupled to tandem mass spectrometry; N° cases: Number of confirmed cases; N/A: not available; NB: newborn; NGS: next-generation sequencing; Pop: description of the population screened; Ref: references used; Scr: Screening assay; tNGS: targeted next-generation sequencing; UEA: United Emirates Arab, WES: whole exome sequencing.

symptoms affecting the brain, endocrine system, heart, gastrointestinal tract, uterus, skin, and immune system that differ based on age of onset. The disorder is caused by a pathological expansion of over 50 CTG repeats in the *DMPK* gene. MD1 onset occurs earlier with each successive generation, which typically happens with maternal transmission. The most severe form, congenital MD1, occurs in 15% of cases. Patients present with severe generalized weakness at birth, respiratory distress, hypotonia, and feeding difficulties. Affected infants may experience delayed cognitive and motor development, intellectual disability, and autism spectrum disorder, with the physical symptoms potentially leading to fatal outcomes. The incidence ranges from 0.5 to 1.8 per 100,000.

Oligonucleotide-based agents and gene therapies have been tested in pre-clinical models and are currently in clinical development. Nevertheless, no specific disease-modifying treatments are currently approved. Management primarily involves monitoring for complications and providing assistive devices, hormone therapy, and pain management.

We identified an article describing a pilot project in the USA that utilized deidentified dried blood spots to determine the prevalence of MD1. The study found a prevalence rate of 1 in 21,100.¹¹² Table 5 summarizes this article.

Rapid genomic diagnosis

Exome and genome sequencing is increasingly used in neonatal intensive care units to diagnose and treat critically ill infants, but the gene panels used for these purposes are poorly reported and therefore little is known regarding the efficacy of this type of testing. The conventional diagnostic approach based on hypotheses might not be effective in these situations, as clinical manifestations during the neonatal period are often nonspecific. Moreover, phenotypes linked to reduced survival in numerous genetic disorders may go unrecognized due to early mortality. In subjects with NMDs, exome and genome sequencing can enable diagnosis to be made. We identified articles reporting two pilot projects in the USA,^{113,114} two in India,^{115,116} two in China,^{117,118} one in South Korea,¹¹⁹ one in Sweden,¹²⁰ one in the United Emirates Arab,¹²¹ and one in Belgium¹²² that evaluated use of genetic testing for diagnosis of NMDs.

Five teams have reported unique cases diagnosed thanks to genetic testing.^{113,115,116,120,121} These diagnoses enabled either early treatment, as was the case for patients with myopathy in Shwachman-Diamond syndrome, a neonatal-onset *MYH2* hereditary myosin myopathy, and congenital hypomyelinating neuropathy, or explained early death (as was the case for a patient with a severe perinatally lethal neuromuscular form of glycogen storage disease IV).¹¹⁶ Five teams have targeted specific populations: hypotonic children, children of low birth weight compared with gestational age, or children with seizures, recruited from neonatal intensive care units, paediatric intensive care units and paediatric neurology

units.^{114,117–119,122} In these studies, genetic testing used targeted panels or more extensive whole exome or whole genome sequencing with the goal of evaluating diagnostic speed and accuracy. In the specific targeted populations, the diagnoses yield was between 27 and 63%, with peripheral hypotonia disorders, Krabbe disease, cerebrotendinous xanthomatosis, SMA, or congenital myasthenic syndrome identified Table 6.

Discussion

Our literature search identified 87 articles describing 82 early identification programs for neuromuscular diseases: 72 studies evaluated NBS programs for seven NMDs and ten studies focused on early genetic diagnosis for various diseases including NMDs. The fast-growing number of peer-reviewed papers in this field illustrates the growing understanding of the importance of early, and ideally pre-symptomatic, identification of patients with treatable conditions such as X-ALD, SMA, Pompe disease, and DMD. These diseases can be diagnosed with a simple biochemical test or straightforward genetic test. Congenital myasthenic syndrome is more difficult to diagnose, and we anticipate similar issues in limb-girdle muscular dystrophies for which treatments are in development but no simple and specific tests are available.

When there are no sensitive biochemical assays nor specific hotspot mutations (such as the deletion of exon 7 of *SMN1* in patients with SMA), which is the case for the majority of NMDs, screening for these disorders will not be compatible with the biochemical methods used in current NBS programs. The fast-paced development of therapies also necessitates the ability to quickly add a gene to a genomic NBS program to ensure that patients are diagnosed as rapidly as possible. Some are hesitant about genomic NBS for several reasons. One primary concern is the potential for false positives or uncertain results, which can cause unnecessary stress and anxiety for families. Additionally, the interpretation of genetic data is complex, and not all detected variants are fully understood, leading to potential misdiagnoses or overdiagnoses. Enabling quicker diagnoses allows for more timely treatments, preventing long-term complications and significantly improving the effectiveness of interventions. For these reasons, several genomic NBS programs are currently underway that use either using WGS or targeted next-generation sequencing.¹²³ Although the panels differ, all screen for mutations in genes implicated in NMDs such as MLD. Parents appear positive about these first genomic NBS programs.¹²⁴ As the cost of adding a new disease is not a limitation when WGS is employed, we expect that many NMDs will be added to the list of diseases evaluated in NBS programs over the next few years. As both genomic NBS and early diagnostic testing will eventually utilize WGS, it is foreseeable that raw data of WGS performed for NBS could be in the future re-analysed for diagnosis in a child presenting any serious condition.

This scoping review has certain limitations. First, only one database (Medline) was consulted. We also restricted

our search to original articles; conference presentations were not considered. Data presented at neuromuscular disease conferences are likely to be published quickly, however, as the neuromuscular world is undergoing a period of transition due to the recent approval of effective treatments.

Conclusion

Since 2021, newborn screening for NMDs, notably in X-ALD, SMA, and Pompe disease, have been incorporated in routine NBS programs. Even for diseases where treatment is currently not life-changing, such as Krabbe disease, new NBS programs continue to be implemented, especially in the USA. The use of genetic diagnostic tests does not yet appear to be widespread, or at least not widely reported. The genomic newborn screening programs currently underway will undoubtedly be a lever for rapid development. Rapid incorporation of tests into routine NBS will be critical as new treatments for NMDs become available.

Abbreviation

DMD	Duchenne muscular dystrophy
EMA	European Medicines Agency
FDA	Food and Drug Administration
MDI	Myotonic dystrophy I
MLD	Metachromatic leukodystrophy
NBS	Newborn screening
NMD	neuromuscular disease
RUSP	Recommended Uniform Screening Panel
SMA	spinal muscular atrophy
USA	United States of America
WGS	whole genome sequencing
X-ALD	X-linked adrenoleukodystrophy

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Data availability

The data supporting the findings of this study are available within the article and/or its supplementary material.

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