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Overview of Neuromuscular Disorder Molecular Diagnostic Experience for the Population of Latvia

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Abstract

Background and Objectives

Genetic testing has become an integral part of health care, allowing the confirmation of thousands of hereditary diseases, including neuromuscular disorders (NMDs). The reported average prevalence of individual inherited NMDs is 3.7–4.99 per 10,000. This number varies greatly in the selected populations after applying population-wide studies. The aim of this study was to evaluate the effect of genetic analysis as the first-tier test in patients with NMD and to calculate the disease prevalence and allelic frequencies for reoccurring genetic variants.

Methods

Patients with NMD from Latvia with molecular tests confirming their diagnosis in 2008–2020 were included in this retrospective study.

Results

Diagnosis was confirmed in 153 unique cases of all persons tested. Next-generation sequencing resulted in a detection rate of 37%. Two of the most common childhood-onset NMDs in our population were spinal muscular atrophy and dystrophinopathies, with a birth prevalence of 1.01 per 10,000 newborns and 2.08 per 10,000 (male newborn population), respectively. The calculated point prevalence was 0.079 per 10,000 for facioscapulohumeral muscular dystrophy type 1, 0.078 per 10,000 for limb-girdle muscular dystrophy, 0.073 per 10,000 for non-dystrophic congenital myotonia, 0.052 per 10,000 for spinobulbar muscular atrophy, and 0.047 per 10,000 for type 1 myotonic dystrophy.

Discussion

DNA diagnostics is a successful approach. The carrier frequencies of the common *CAPN3*, *FKRP*, *SPG11*, and *HINT1* gene variants as well as that of the *SMN1* gene exon 7 deletion in the population of Latvia are comparable with data from Europe. The carrier frequency of the *CLCN1* gene variant c.2680C>T p.(Arg894Ter) is 2.11%, and consequently, congenital myotonia is the most frequent NMD in our population.

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Genetic testing has become an integral part of health care, allowing the confirmation of thousands of hereditary diseases, including neuromuscular disorders (NMDs). In a single decade, genetic tests have been established as a reliable tool for routine diagnostics and are undoubtedly effective for diagnosing persons with alleviated and atypical symptoms, or those in the presymptomatic period, and are useful for diagnosing multiple inherited diseases in one person. Furthermore, genetic results have recently provided the option of custom-tailored management or treatment of genetic disorders, particularly NMDs. Targeted gene therapy options were another breakthrough for patients with NMDs. Recently, a number of patients with spinal muscular atrophy (SMA) and Duchenne muscular dystrophy were able to receive gene therapy and consequently had substantially better outcomes,[1,2] emphasizing the need for a precise and timely genetic diagnosis.

The average prevalence of all inherited NMDs is 37–49.9[3] per 100,000. This number varies greatly in the selected populations after applying population-wide studies. In 2017, a meta-analysis of epidemiology of SMA was performed, and reported an average estimated prevalence of 1–2 per 100,000 and an incidence of 1 per 10,000 live births.[4] In 2021, significantly different prevalence values were calculated using an SMA genetic test in newborn screening pilot studies. The prevalence was as high as 1 in 6,910 in Germany[5] and as low as 1 in 17,947 in the New England Newborn Screening program in Massachusetts, USA.[6] Another example is myotonic dystrophy type 1, which has a calculated prevalence of 10.4 per 100,000.[3,7] In 2021, using dried blood spot cards from the newborn screening program in the state of New York, Johnson et al.[8] calculated a ×5 higher prevalence of 4.76 per 10,000 births for the disease.

Historically, the performance of muscle biopsy analysis with standard histochemistry, supplemented by additional immunocytochemical studies and/or Western blot analysis, was paramount in the diagnostic workup for patients with neuromuscular diseases. In the absence of this diagnostic tool, since 2008, there has been a natural shift toward genetic tests as a first-line option for NMDs in our population. Our objective was to evaluate the effect of genetic analysis as the first-tier test in patients with NMD in the population of Latvia.

Methods

Participants

Data of molecular testing from the medical records of all patients suspected to have a hereditary NMD were included in this observational retrospective study. Two main centers in Latvia offered genetic diagnosis and counseling for the Latvian population.

Demographic and genetic data from the Medical Genetics Clinic, Children's Clinical University Hospital in 2015–2020, and the Latvian Biomedical Research and Study Center in 2008–2020 were included in this study. The inclusion start date differed between the 2 centers because routine DNA diagnostics for genetic diseases only became available at the Medical Genetics Clinic, Children's Clinical University Hospital in 2015. Genetic tests were performed at both clinical and private laboratories as well as research centers nationally and abroad. These included neuromuscular disease gene panels, whole-exome sequencing (WES), whole-genome sequencing, and nucleotide repeat expansion/contraction genetic tests.

In addition, reports (2008–2020) of myotonic dystrophy type 1, spinobulbar muscle atrophy, and SMA were included from the respective national laboratories directly (eTable 1, links.lww.com/NXG/A529).

For the population data, the control group consisted of 190 randomly selected healthy, unrelated individuals from the Genome Database of the Latvian Population, who represented the general population. For the population screening of *SMN1* and *SMN2* copy numbers, DNA samples of 282 healthy volunteers, regardless of their ethnicity, were obtained from the Genome Database of the Latvian Population.[9]

Standard Protocol Approval, Registration, and Patient Consent

Data collection was performed in accordance with the permission Nr. 27 issued by the Central Medical Ethics Committee of Latvia. The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

All participants or their parents/legal guardians (in the case of children younger than the age of consent) signed an informed consent form if included in the research project.

Statistical Analysis

Demographic data were obtained from the Central Statistical Bureau of Latvia.[10] For SMA and dystrophinopathies, which present during early childhood, birth prevalence was calculated. SMA tests confirmed 27 positive cases among children born in 2008–2020; the number of newborns in this period was 267,713. The prevalence of dystrophinopathy was calculated, with 13 affected boys born in 2015–2020 of 62,284 newborn baby boys in this period. In December 2020, the point prevalence of the following adult-onset diseases was calculated in the population of Latvia (n = 1,907,675): facioscapulohumeral muscular dystrophy (FSHD), myotonia congenita, limb-girdle muscular dystrophy, spinobulbar muscular atrophy (male population), and myotonic dystrophy type 1. In addition, the 95% confidence interval (CI) was calculated.

Allelic Frequency

All variants, but not the *SMN1*/*SMN2* exon 7 copy number, were analyzed by direct sequencing in the control group to obtain an allelic frequency in the population. *SMN1*, *SMN2*,

Table 1 Identified Variants in Patients With Neuromuscular Disease

Gene	Variant identified in a patient	Gene	Variant identified in a patient
ACTA1	c.1106C>T p.(Pro369Leu)	*DMD*	Deletion 44 exon
AR	(CAG)–50 repeats		Duplication 56–57 exons
	(CAG)–48 repeats		Deletion 45–48 exons
	(CAG)–54 repeats		Deletion 8–12 exons
	(CAG)–47 repeats	*DNAJB6*	c.279C>G, p.(Phe93Leu)
	(CAG)–53 repeats	*DYSF*	c.4135T>C, p.(Cys1379Arg)
CACNA1A	**c.(784+1_785-1)_(978+1_979-1)del**		c.5668-824C>T
CAMTA1	**c.2500_2501del, p.(Ser834Glnfs*67)**	*FKRP*	**c. 204_206del**
CAPN3	c.550del p.Thr184fs (n = 3)		c.826C>A, p.(Leu276Ile) (n = 7)
	c.643T>C p.(Ser215Pro) (n = 2)	*LAMP2*	**c.(64+1_65-1)_(864+1_865-1)dup**
	c.1043del p.(Gly348fs)	*LMNA*	c.1357C>T, p.(Arg453Trp)
	c.1079G>T, p.(Trp360Leu)	*MT-TK*	m.8344A>G
	c.1333G>C p.(Gly445Arg) (n = 2)	*MUSK*	**c.1274_1278del, p.(Glu425Alafs*30)**
CLCN1	c.1746-20C>G (n = 3) c.1437_1450del p.(Pro480fs) (n = 2)	*MYBPC1*	**c.1875>T, p.(Arg625Ser)** c.742G>A, p.(Glu248Lys)
	c.1438C>T p.(Pro480Ser) (n = 2)	*MYH7*	c.3830G>C, p.(Arg1277Pro)
	c.1649C>T p.(Thr550Met)	*NEB*	c.2211+5G>A
	c.2680C>T p.(Arg894Ter) (n = 19)		**c.18665delG, p.(Cys6222Phefs)**
COL6A1	c.930+189C>T		c.19324C>T, p.(Arg6442Trp)
COL6A3	c.7447A>G p.(Lys2483Glu)	*POMGNT1*	**c.448G>C, p.(D150H)**
	c.8074delT p.(Tyr2692MetfsTer15)		c.1324C>T, p.(Arg442Cys)
DCTN1	**c.1740G>T, p.(Gln580His)**		c.1539+1G>A
DDC	c.476C>T, p.(Ala159Val)		c.1814G>A, p.(Arg605His)
DMD	c.(31+1_32-1)_(93+1_94-1)dup	*POMT1*	c.229+2T>C
	c.188del, p.(Pro63Glnfs*12)		**c.512T>G, p.(Leu171Arg)**
	c.572C>A, p.(Ser191Ter)	*PRG4*	c.6_7dup, p.(Trp3Tyrfs*17)
	c.1399dup, p.(Thr467Asnfs*16)	*SCN11A*	c.665G>A, p.(Arg222His)
	c.(?_-1)_(3786+1_3787-1)del	*TTN*	c.99673+1G>A
	c.4729C>T, p.(Arg1577Ter)	*UNC80*	**c.2707G>A, p.(Ala903Thr)**
	c.5773G>T, p.(Glu1925Ter)		**c.3356G>C, p.(Ser1119Thr)**
	c.6292C>T, p.(Arg2098Ter)		
	c.6420del, p.(Lys2140Asnfs*23)		
	c.8120delA, p.(A2708Lfs*18)		
	c.8443C>T, p.(Gln2815Ter)		
	c.8944C>T, p.(Arg2982Ter)		

Abbreviation: DMD = dystrophinopathy.
In bold are represented novel genetic variants, which were absent from ClinVar or LOVD databases.

Table 2 Prevalence per 10,000 for SMA, DMD, FSHD1, Congenital Myotonia, LGMD, DM1 and BSMA

Disorder/group of disorder	No. of patients (n)	Present study birth and point prevalence per 10,000	95% confidence interval, present study	Published data prevalence per 10,000
SMA	27	1.01	0.66–1.47	0.1–1.0[i]
DMD	13	**2.08**	1.11–3.57	1.98[j]
FSHD1	15	**0.079**	0.044–0.113	1.2[a]
Congenital myotonia	14	**0.073**	0.040–0.123	0.075[b]–0.9[k]
LGMD	15	**0.078**	0.044–0.113	0.52[c]
DM1	9	**0.047**	0.022–0.90	1.04[d]–4.76[e]
BSMA	5	**0.052**[f]	0.016–0.122	0.228–1.5[g,h]

Abbreviations: BSMA = bulbospinal muscular atrophy; DM1 = myotonic dystrophy type 1; DMD = dystrophinopathy; FSHD1 = facioscapulohumeral muscular dystrophy, type 1; LGMD = muscular dystrophy, limb-girdle; SMA = spinal muscular atrophy.
[a] Reference 18.
[b] Reference 19.
[c] Reference 20.
[d] Reference 7.
[e] Reference 8.
[f] Reference 21.
[g] Reference 22.
[h] Reference 23.
[i] Reference 4.
[j] Reference 15.
[k] Reference 24.

and *RPP30* concentrations were measured using a Bio-Rad QX200 Droplet Digital PCR system. QuantaSoft Analysis Pro (Bio-Rad) was used for droplet cluster classification and Poisson function applications to calculate absolute and relative *SMN1*, *SMN2*, and *RPP30* copy numbers. Details of the PCR and ddPCR assays are available in Supplement (eMethods, links.lww.com/NXG/A529).

Data Availability

Anonymized data not published within this article will be made available on request from any qualified investigator. The statistical analysis plan is available in the Supplement.

Results

The disease-associated variant was found in 62 of 137 patients with a suspected NMD seen at the Children's Clinical University Hospital and in 59 of 151 patients seen at the Latvian Biomedical Research and Study Center. Altogether, diagnosis was confirmed in 100 unique cases of 267 persons tested (there was an overlap of 21 patients because analysis was initiated by 1 center, and the patient was transferred to the other center), with a total detection rate of 37%. Independently, 3 genetic laboratories in Latvia reported their diagnostic findings from 2008 to 2020, with SMA confirmed in 47 of 254 persons tested (27 of whom were born in 2008–2020), myotonic dystrophy type 1 identified in 9 individuals (data from 2 laboratories), and 5 unrelated male patients diagnosed with Kennedy disease. NMD diagnosis was confirmed in 153 persons in total, and this will be further analyzed in this study (eTable 1, links.lww.com/NXG/A529). All the identified unique genetic variants are listed in Table 1, and the number of individual cases is denoted in brackets. If the pathogenic variant was discovered in multiple members of the same family, it was considered a single case. Two and 3 different genetic disorders were simultaneously discovered, each in a single patient, which considerably complicated their clinical phenotype.

The calculated birth prevalence was 1.01 per 10,000 (95% CI: 0.66–1.47) for SMA and 2.08 per 10,000 (newborn male population, 95% CI: 1.11–3.57) for dystrophinopathies. The point prevalence was 0.079 per 10,000 (95% CI: 0.044–0.113) for FSHD type 1, 0.073 per 10,000 (95% CI: 0.040–0.123) for nondystrophic congenital myotonia, 0.078 per 10,000 (95% CI: 0.044–0.113) for limb-girdle muscular dystrophy (LGMD), 0.047 per 10,000 (95% CI: 0.022–0.090) for type 1 myotonic dystrophy, and 0.052 per 10,000 male population (95% CI: 0.016–0.122) for spinobulbar muscle atrophy. A summary of all point prevalence values and their comparison with published data is provided in Table 2. Patients with LGMD were divided into the following subgroups: LGMD R1 calpain3-related (n = 6), LGMD D1 DNAJB6-related (n = 4), LGMD R2 dysferlin-related (n = 1), and LGMD R9 dystroglycan-related (n = 4).

Reoccurring variants of the *CAPN3*, *CLCN1*, and *FKRP* genes were identified in unrelated individuals, and their allelic frequencies were calculated using samples from the Genome Database of the Latvian Population. Allelic frequencies of these variants are listed in Table 3. In addition, we determined the allelic frequencies of common variants of the *HINT1* and *SPG11* genes, which are responsible for neuromyotonia/axonal neuropathy and autosomal recessive spastic paraplegia 11, respectively, because of their observed frequencies in our population. The obtained data are listed in Table 3. In

Table 3 Allele Frequency for the Selected Variants

Gene	Variant	AF in European population (non-Finnish),[a] %	AF in Estonian population,[a] %	AF in Latvian population, present study,%
CAPN3	c.1746-20C>G	0.46	1.47	2.37[c]
	c.550del p.(Thr184fs)	0.04	0.21	0.16[b]
CLCN1	c.2680C>T p.(Arg894Ter)	0.34	1.93	2.11[c]
FKRP	c.826C>A p.(Leu276Ile)	0.23	0.19	0.30[b]
HINT1	c.110G>C p.(Arg37Pro)	0.046	0.31	1.10[c]
SPG11	c.2431C>T p.(Gln811Ter)	0.0078	0.15	0.53[c]

Abbreviation: AF = allele frequency.
[a] gnomADv2.1.1.
[b] Reference 25.
[c] Present study.

addition, we determined the copy numbers of *SMN1* and *SMN2* exon 7 because a homozygous deletion of *SMN1* exon 7 is a common cause of SMA.[11] We identified 6 individuals carrying 1 copy of *SMN1* exon 7; therefore, the estimated carrier frequency in the population of Latvia was 2.1% or 1 of 47 individuals. The frequencies of 2, 3, and 4 copies of the *SMN1* gene were 94.7%, 2.8%, and 0.4%, respectively. The *SMN2* copy number ranged from 0 to 3. The frequencies of 0, 1, 2, and 3 copies of the *SMN2* gene were 6.4%, 37.2%, 54.3%, and 2.1%, respectively (Table 4).

Discussion

In the past decade, medical care of patients with NMD in Latvia has purposefully shifted toward using DNA diagnostics as the first-line confirmatory test. After essential clinical, electrophysiologic, and biochemical investigations have been performed, all patients with a suspected NMD are referred to clinical geneticists. Larger and more informative tests are preferred over a sequential diagnostics approach. Exceptions include situations when individual tests for facioscapulohumeral muscle dystrophy or myotonic dystrophy are primarily ordered. In this study, we summarize more than 10 years of experience using the current approach.

The profile of the identified disorders combines patients carrying frequent European pathogenic variants of the *FKRP, CAPN3*, and *CLCN1* genes, as well as some ultrarare cases, such as those carrying the *MYBPC1* pathogenic variant that causes congenital myopathy and myogenic tremor. The diversity of these results confirms the necessity of approaches using larger gene panels or WES. However, the numbers of identified patients carrying the *FKRP* gene variant c.826C>A p.(Leu276Ile) (n = 7) and the *CLCN1* gene variant c.2680C>T p.(Arg894Ter) (n = 19) warrants a discussion about testing for these single genetic changes before gene panel testing or WES is performed in patients with relevant clinical symptoms or transferring this step in medical care to additionally trained specialists prior to genetic counseling. This suggestion is supported by the identified allelic frequency of the *CLCN1* gene variant c.2680C>T p.(Arg894Ter) in the population of Latvia (2.11%), which is higher than that in the non-Finnish European population (0.34%) and the population of Estonia (1.93%), as reported in the gnomAD database.[12] Allelic frequency data in conjunction with the already identified patients led us to hypothesize that congenital myotonia caused by the *CLCN1* gene variant c.2680C>T p.(Arg894Ter) is the most frequent NMD in Latvia.

The situation with calpainopathy in our population is complicated by the presence of the frequent allele c.1746-20C>G, with conflicting interpretation of its pathogenicity.[13] This allele was identified in 3 cases in a trans compound heterozygous state with another pathogenic/likely pathogenic recessive variant. Until further studies are performed to confirm or refute its role in the development of limb girdle muscle dystrophy, patients with this allele require individual case-by-case management by clinical geneticists together with an NMD team. While the high frequencies of the pathogenic variants

Table 4 Spinal Muscular Atrophy Carrier Screening Results

Gene	Copy number	Frequency, %
SMN1	0	0
	1	2.1
	2	94.7
	3	2.8
	4	0.4
SMN2	0	6.4
	1	37.2
	2	54.3
	3	2.1
	4	0

c.826C>A p.(Leu276Ile) of the *FKRP* gene and c.550del p.(Thr184fs) of the *CAPN3* gene in Latvia are similar to those in other European populations, the frequency of the *CAPN3* gene variant c.1746-20C>G is significantly higher at 2.37%. Nevertheless, the current data do not allow us to draw conclusions about its role in the pathogenesis of calpainopathy.

Since 2008, DNA diagnostics have helped to diagnose 37% of our patients with NMD. This detection rate is not directly compatible with those in previous publications because the genetic tests mentioned in those studies have evolved over time. Only small gene panels and limited copy number analysis were available 10 years ago, while WES, enriched with mitochondrial genome analysis, can be the first-line option nowadays. Nevertheless, very similar results were reported by Harris et al. in cohorts of patients with LGMD for whom WES was performed instead of sequential gene panel testing. They were able to genetically diagnose 37% of patients, and although the total diagnostic yield of standard sequential testing was not much lower at 33%, it was less timely.[14]

To ascertain the validity of our results, point prevalence was calculated, and the results were compared with those from published studies. Pediatric patients are a priority for genetic counseling and testing. Some of the most common childhood-onset NMDs in our population were SMA and dystrophinopathies, with a birth prevalence of 1.01 per 10,000 and 2.08 per 10,000 (male population), respectively, which are comparable with data published by other countries.[5,15,16] The carrier frequency of the *SMN1* exon 7 deletion varies between 1 of 40 and 1 of 100 individuals, depending on geographic origin and ancestry.[17] The carrier frequency in the population of Latvia was 1 of 47 individuals or 2.1%, which matches with the general European genetic landscape. SMA and dystrophinopathies are the most common disorders, and specialists are well-trained to recognize them. Accessibility of gene therapy for these diseases intensifies the pressure for early diagnosis, and the industry offers regular training for specialists to raise awareness of these disorders.

The unavailability of genetic testing on a regular basis for adults before 2015 left this group of patients in a particularly sorrowful situation. This is well represented in our disease prevalence calculations; the number of identified patients with myotonic dystrophy type 1 and FSHD is at least 10 times lower than in published studies from Europe and the USA.[8,18] A possible explanation for this is the complicated stepwise genetic test of the *DMPK* gene and the difficulties justifying further investigation of cases with inconclusive genetic screening results. Access to myotonic dystrophy type 1 and FSHD confirmatory level tests in national laboratories would facilitate the diagnosis of patients, allowing all involved specialists, cardiologists, ophthalmologists, and, most importantly, neurologists to order these tests directly. The phenotypic variability observed as well as anticipation makes identifying these diseases more difficult.

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Disclosure

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