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Duchenne muscular dystrophy (DMD) is a severe, progressive, X-linked, recessive neuromuscular disease that affects approximately 1 in 5000 live male births.[1] Patients with DMD experience progressive muscle weakness, owing to the absence of functional dystrophin protein, and typically experience delayed walking, difficulty running or climbing stairs, and frequent falls.[2,3] However, several nonmotor signs and symptoms (eg, behavioral issues, neurocognitive deficits, and speech delay) also can be associated with the disease.[3] Boys with DMD are generally diagnosed between 4 and 5 years of age, when their physical development begins to diverge more clearly from that of their peers,[3-6] and, with corticosteroid treatment, typically lose ambulation at a median age of about 12.0-14.0 years.[7] However, this can vary by country.

The accurate and early diagnosis of DMD plays a crucial role in the effective management of patients: it has the potential to lead to earlier intervention; appropriate genetic counseling; treatment with mutation-specific therapies (where applicable); and appropriate assignment to clinical trials. However, reports indicate that significant delays in diagnosis of DMD persist.[4-6,8] It has been shown that in the absence of a family history of DMD, there is a delay of approximately 1 year from onset of earliest symptoms to the first assessment by a healthcare professional.[4] A further delay of approximately 1 year from this first assessment to referral to a neurologist or a neuromuscular specialist is observed (mean age at diagnosis ± SD: 4.9 ± 1.7 years).[4] Findings by Vry et al[6] suggest that this could be shorter, with a mean delay of just over 1 year from first symptoms to diagnosis (mean delay ± SD: 1.3 ± 1.8 years). Overall, these findings suggest that, on average, there has been little to no reduction in the age at diagnosis for patients with DMD over the last 30 years.[9,10]

Once DMD is suspected, genetic testing is required to obtain a complete diagnosis.[3,11,12] However, results from a recent survey of 41 delegates from Europe, Turkey, and India (primarily child neurologists and clinical/molecular geneticists) revealed that there may be issues relating to the genetic diagnosis for DMD. For example, although 100% of delegates understood the importance of genetic testing for DMD, more than 10% did not perform additional genetic tests if deletion/duplication testing was negative.[11] Survey results from the 2017 and 2018 TREAT-NMD Expert Masterclasses on DMD, attended by more than 100 delegates combined (primarily from pediatrics and neurology backgrounds from 27 and 20 countries, respectively), showed that some delegates experienced difficulties interpreting DMD genetic test results (**Table I**; available at www.jpeds.com) and subsequently were not always aware of whether patients were eligible for treatment with mutation-specific therapies. Together, these issues highlight the need for shorter times to diagnosis for patients with DMD and clearer recommendations for DMD genetic testing to ensure complete genetic assessment is performed to reach an accurate genetic diagnosis. This review, supported by a systematic literature search, presents expert consensus on ways of reducing the time to diagnosis of DMD.

Methods

The Delphi Consensus Initiative presented here is focused on how to reduce the time to diagnosis of DMD. The development process for this initiative is summarized in **Figure 1**. The steering committee comprised 3 experts in the field of human genetics, specializing in the diagnosis of DMD and/or interpretation of genetic mutations: S. F. Nelson (nonvoting chair), A. Aartsma-Rus (voting co-chair), and M. Hegde (voting co-chair). After an initial meeting of the steering committee, 14 experts in the field were invited to form the expert voting panel. The panel comprised primarily medical geneticists specializing in the diagnosis of patients with DMD; however, 2 child neurologists, 1 patient advocate, and 1 genetic counselor also were invited. All members of the expert voting panel and the 2 voting co-chairs voted anonymously on the statements to reach consensus. A systematic literature review was also performed to support development of the consensus statements

BMD	Becker muscular dystrophy
CK	Creatine kinase
DMD	Duchenne muscular dystrophy
MLPA	Multiplex ligation-dependent probe amplification
NBS	Newborn screening
NGS	Next-generation sequencing

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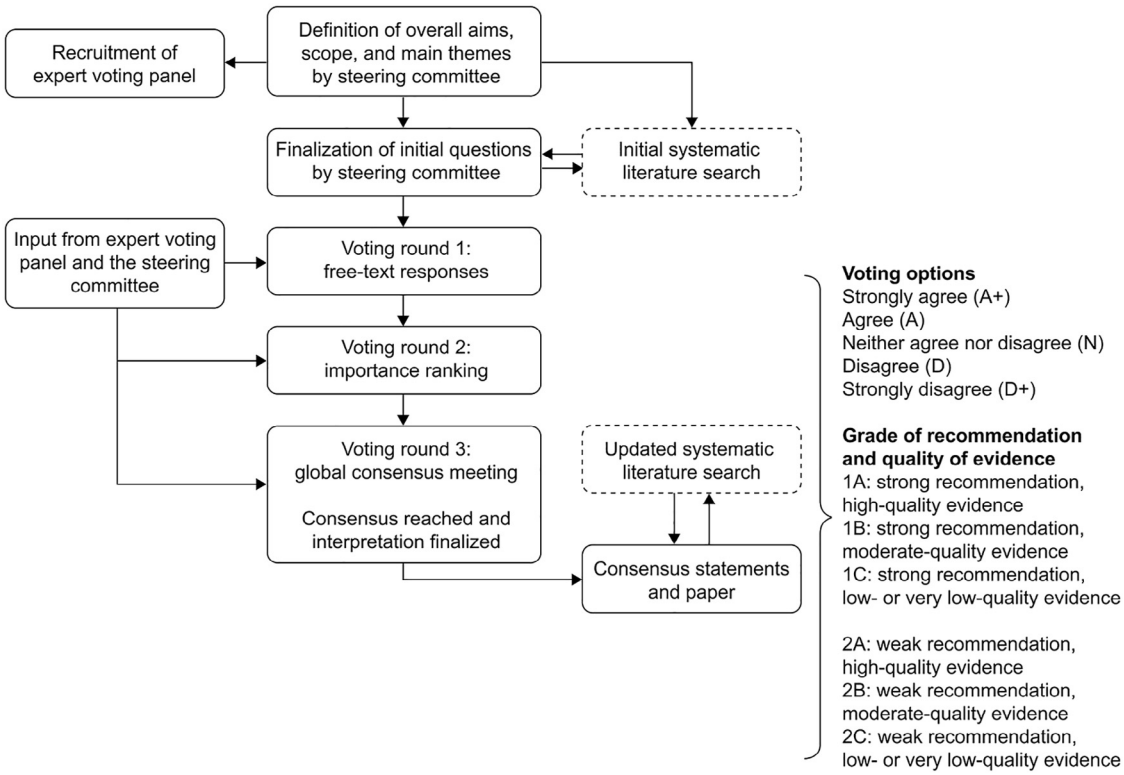

Figure 1. Delphi Consensus Initiative development process following the steps outlined by Rosenfeld et al[13] and summary of the Grading of Recommendations Assessment, Development, and Evaluation process,[14-17] including voting options.

(PRISMA flow diagram; **Figure 2** and **Table II** [available at www.jpeds.com]). The evidence was graded using the Grading of Recommendations Assessment, Development, and Evaluation system (**Figure 1**). Further information regarding the development process is provided in the **Appendix** (available at www.jpeds.com). The grading of evidence for the statements was reviewed and agreed on by the expert voting panel (**Table III**).

Discussion

Consensus Statements

The 15 consensus statements are presented in **Table III**. A summary of the supporting information is provided herein, with a full discussion included in the **Appendix**. Several other more general statements were discussed, but only those most pertinent to reducing the time to diagnosis of DMD are presented here.

Section 1: Reducing the Time to Diagnosis of DMD

Signs and symptoms of DMD (motor and nonmotor) are presented in the 2018 care guidelines for DMD.[3] The consensus group agreed on a series of motor signs and symptoms (**Table III**) that are typically observed in patients with DMD (supported by the literature; **Statement 1**). It also should be noted that patients are sometimes referred because of elevated alanine transaminase or aspartate transaminase levels,[3] and that this finding also should require a high index of suspicion from physicians. The consensus group agreed on a number of nonmotor signs and symptoms that may also act as indicators of DMD (autism spectrum disorder, delayed speech/cognitive deficits, and gross motor delay; **Statement 2**) but are not always associated with the disease and may need wider clinical assessment. As such, these symptoms may sometimes be overlooked, and as a result, patients could be referred incorrectly, for example to a physical, occupational, or speech therapist.[4] As a result, the time to diagnosis for these patients can be delayed. The consensus group therefore agreed that for patients presenting with cognitive or developmental deficits, DMD should be considered as part of the differential diagnosis.

Patients presenting with the motor and nonmotor signs and symptoms of DMD as per **Statements 1** and **2** should immediately have their serum creatine kinase (CK) levels tested and be referred to a child neurologist or a neuromuscular specialist.[3] A marked increase in serum CK, defined as >2000 IU/L,[31] should prompt further investigation for DMD. However, elevated CK levels within the range of approximately 500-1200 U/L (1.5 times the upper limit of normal for men), even if asymptomatic, may be indicative of other neuromuscular disorders that require further assessment.[103] It has been shown that once a patient has had a serum CK test, the time to reach a complete diagnosis of DMD is relatively short (mean age ± SD [range] at first CK test, 4.7 ± 1.7 [0.3-8.6] years, n = 151; and at complete diagnosis, 4.9 ± 1.7 [0.3-8.8] years, n = 154).[4]

Table III. Summary of statements and recommendations for reducing the time to diagnosis of DMD

Consensus statements	Consensus vote	Strength of recommendation	Level of evidence*	Final GRADE score†	Key supporting evidence
Section 1: Reducing the time to diagnosis of DMD					
Statement 1: The following signs, symptoms, and characteristics should be considered typical indicators of DMD: calf hypertrophy (pseudohypertrophy); delayed walking; difficulty climbing/descending stairs; difficulty rising from the floor; difficulty running/walking; elevated serum CK levels (including elevated ALT and AST); a family history of DMD; frequent falls; Gowers' sign; male sex; and muscle weakness.	A + = 93%; A = 7%	Strongly in favor	Moderate	1B	[3,4,8,18-22]
Statement 2: Autism spectrum disorder, delayed speech/cognitive deficits, and gross motor delay can be indicators of DMD but are not always associated with the disease.	A + = 93%; A = 7%	Strongly in favor	Moderate	1B	[3,4,8,18,21,23-30]
Statement 3: After initial presentation, patients with suspected DMD should have their serum CK levels tested and be referred to a specialist (a child neurologist or neuromuscular specialist).	A + = 93%; A = 7%	Strongly in favor	Moderate	1B	[3,4,8,18,31-33]
Statement 4: A lack of awareness of DMD and associated symptoms by the healthcare professional and long waiting times to see a specialist are the primary factors leading to a delay in initial diagnosis.	A + = 67%; A = 33%	Strongly in favor	Low	1C	[4,8]
Statement 5: Genetic testing is crucial for obtaining a complete diagnosis of DMD, and should be considered the gold standard.	A + = 94%; A = 6%	Strongly in favor	Low	1C	[3,11,18] and expert opinion
Statement 6: In the majority of cases, a complete genetic diagnosis can be made using MLPA or CGH to detect deletions or duplications in the *DMD* gene.	A + = 88%; A = 6%; N = 6%	Strongly in favor	Moderate	1B	[3,11,12,18,34-44] and expert opinion
Statement 7: If exon-level deletions/duplications in the *DMD* gene are not identified, small-scale mutations (by sequencing of exons and flanking regions) should be tested for as the next step.	A + = 93%; A = 7%	Strongly in favor	Low	1C	[3,11,35] and expert opinion
Statement 8: Muscle biopsies with dystrophin staining are generally not needed to obtain a complete diagnosis of DMD, unless DNA testing is negative.	A + = 73%; A = 27%	Strongly in favor	Low	1C	[3,11,12,35,43,45-54] and expert opinion
Statement 9: Delays in the initial clinical diagnosis/referral to a specialist, the sequential nature of the genetic testing process, and incomplete or nonexhaustive genetic testing should be addressed in order to prevent delays in reaching a complete genetic diagnosis for patients with DMD.	A + = 93%; A = 7%	Strongly in favor	Low	1C	[4,8,11,19] and expert opinion
Section 2: Recommendations for next steps following a suspected DMD diagnosis					
Statement 10: Patients with signs and symptoms of DMD and elevated serum CK levels should be referred for genetic testing to either a clinical geneticist or a neuromuscular specialist.	A + = 100%	Strongly in favor	Low	1C	[3,55,56] and expert opinion
Statement 11: A medical/clinical geneticist, a child neurologist, or a neuromuscular specialist should request the genetic test and should provide clinical information relevant to the diagnosis as part of the sample submission to the clinical genetics laboratory, and the genetic diagnostic test should be performed by an accredited laboratory.	A + = 100%	Strongly in favor	Low	1C	[12,57] and expert opinion
Statement 12: Educational meetings for physicians and laboratory specialists on topics relating to the genetic diagnosis of DMD would help to improve the understanding of genetic test reports and the interpretation of genetic test results.	A + = 93%; A = 7%	Strongly in favor	Low	1C	[58-62] and expert opinion
Statement 13: Genetic testing is necessary to inform carrier testing, family planning, genetic counseling, prognosis and optimal management strategies, natural history data gathering, and prenatal diagnosis.	A + = 100%	Strongly in favor	Low	1C	Carrier testing/family planning[11,63-73]; genetic counseling[71,74]; prognosis/management[23,75-78]; natural history[7,79,80]; prenatal diagnosis[12,31]; and expert opinion
Statement 14: After a patient receives a complete genetic diagnosis of DMD, it is mandatory that carrier testing of the mother and other at-risk female family members be offered with appropriate pre- and postgenetic counseling (information regarding germline mosaicism and de novo mutations should also be offered). Similarly, testing of other at-risk male family members should also be offered.	A + = 100%	Strongly in favor	Moderate	1B	[11,12]; carrier testing[64-66,68-70,73,81-90]; germline mosaicism/de novo mutations[91-95]; family planning/genetic counseling[71,74]; and expert opinion
Statement 15: When a family history is present, pre-symptomatic CK testing and prenatal testing can lead to earlier detection and thus management of DMD, which is important for family planning.‡	A + = 87.5%; A = 12.5%	Strongly in favor	Low	1C	[1,12,31,96-102] and expert opinion

ALT, alanine aminotransferase; *AST*, aspartate aminotransferase; *CGH*, comparative genome hybridization; *CK*, creatine kinase; *DMD*, Duchenne muscular dystrophy; *GRADE*, Grading of Recommendations Assessment, Development, and Evaluation.

*The level of evidence for most of the statements was graded as either low or moderate, owing to the fact that most of the studies included here are observational in nature rather than randomized controlled trials (due to the nature of this initiative). When there were multiple corroborative supporting observational studies, we have selected "moderate" for quality of evidence.

†Consensus: A + = strongly agree; A = agree; N = neither agree nor disagree; D = disagree; D + = strongly disagree. Grade of recommendation: 1A = strong recommendation, high-quality evidence; 1B = strong recommendation, moderate-quality evidence; 1C = strong recommendation, low-quality or very low-quality evidence; 2A = weak recommendation, high-quality evidence; 2B = weak recommendation, moderate-quality evidence; 2C = weak recommendation, low-quality or very low-quality evidence.

‡Where applicable depending on country-specific legislation on presymptomatic testing of patients aged ≤18 years.

Indeed, patients who are identified by an incidental finding of elevated CK (before onset of signs and symptoms) can be diagnosed earlier.[8] The consensus group agreed that a lack of awareness of the signs and symptoms of DMD and delays in the time taken to see a specialist are the primary factors contributing to a delay in the initial diagnosis. In support of this, a retrospective chart review of 156 patients without a family history of DMD using the Muscular Dystrophy Surveillance, Tracking and Research Network (MD STARnet) showed, in a subset of patients (n = 127), that 63.8% (81/127) were seen by a pediatrician/family practitioner at their first evaluation and that a CK test was ordered as a result of the first evaluation in only 34.6% (44/127) of cases.[4] This finding indicates a need to increase awareness of DMD among front-line healthcare professionals.

The consensus group agreed that genetic testing is crucial to obtain a complete diagnosis of DMD. The *DMD* gene is one of the largest known human genes (2.2 Mb), containing 79 exons[34,104] with a relatively high mutation rate (~30% of cases are caused by a de novo mutation).[11,45,105] The approximate distribution of mutations in the *DMD* gene is as follows: deletion of 1 or more exons, 68%; duplication of 1 or more exons, 11%; small-scale mutations, 20% (small-scale deletions, 5%; small-scale insertions, 2%; splice-site, 3%; nonsense, 10%; missense, 0.4%); and deep intronic mutations, 0.3%.[35] This distribution is supported by a number of studies.[23,36,37,46,106-110] It is therefore practical to test for *DMD* gene mutations in order of frequency. The consensus group agreed on 3 statements that outline the recommended steps needed to reach a complete genetic diagnosis of DMD (**Figure 3**).[4,35,46] First, multiplex ligation-dependent probe amplification (MLPA) or comparative genome hybridization should be used to screen for deletions or duplications in the *DMD* gene. These tests are recommended because they can screen all 79 exons of the *DMD* gene; however, it should be noted that there are several limitations associated with MLPA. For example, point mutations or polymorphisms along the probe hybridization region can present as single-exon deletions when using this method, and thus a second confirmatory test (usually Sanger sequencing) is required.[11,12,31] However, if the mutation is identified and correlates with the severity of symptoms (eg, DMD or Becker muscular dystrophy [BMD]), no further genetic testing is typically required. If exonic deletions/duplications in the *DMD* gene are not identified, small-scale mutations should be tested for by sequencing exons and flanking intronic regions. If clinical signs and genetic testing are conclusive, in most cases, muscle biopsies are not needed. Nevertheless, if no mutation is detected after DNA analysis, it is possible that the patient may have a large rearrangement, such as an inversion (or translocation in females[11]), a deep intronic mutation that affects splicing, or an alternative diagnosis.[12] Inversions and translocations are challenging to detect with most conventional genetic analyses, because they do not affect copy number.[111] Deep intronic mutations often can be detected by next-generation sequencing if the full genomic sequence of the gene is available[37,112]; however, it can be difficult to predict the impact of intronic mutations (or variations) on mRNA. If a muscle biopsy with dystrophin staining confirms dystrophinopathy, mRNA analysis should be performed to identify any impact on mRNA splicing[11,12,45,113] that escapes detection by both MLPA and DNA sequencing. RNA analysis is also crucial to determine the consequence of the mutation on the mRNA and can be considered for discordant phenotypes. Muscle biopsy remains a relevant diagnostic tool, especially for mutation types in which the mutation is poorly predictive of disease progression (for instance, a deletion of exons 3-7 can cause both BMD and DMD phenotypes), or for determining dystrophin expression in boys with unexpectedly mild disease progression.[11] We note that there are several databases that can help physicians to determine the severity of individual and rare causal mutations in the *DMD* gene (**Appendix**). In addition to consulting these databases, physicians entering new data on genotype and phenotype are warranted to help inform future diagnoses.

The consensus group agreed that delays in the initial clinical diagnosis or referral to a specialist, the sequential nature of the genetic testing process, and incomplete or nonexhaustive genetic testing should be addressed to prevent delays in diagnosis. In addition, if the ordering physician is not sufficiently informed or trained to understand the hierarchy of tests needed to provide a complete genetic diagnosis for DMD, the testing process could be terminated prematurely (as shown elsewhere).[11] It would therefore be helpful if the responsibility for these decisions was integrated with the primary testing laboratory's operating procedures. The laboratory would thus be obligated to contact the ordering physician to discuss the next level of testing required either to obtain a complete genetic diagnosis of DMD or to exclude DMD from the diagnosis.

Section 2: Recommendations for Next Steps Following a Suspected DMD Diagnosis

A patient with signs and symptoms of DMD and elevated CK levels should be referred to a clinical geneticist/neuromuscular specialist during the genetic testing process, because these individuals are best placed to provide accurate interpretation of genetic test results[55] and can help to avoid diagnostic delay (**Table III**).[3]

In 2010, best practice guidelines for the molecular diagnosis of DMD/BMD were developed using a consensus-building approach.[12] More recently, general standards and guidelines for the interpretation of sequence variants have been published by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.[57]

The consensus group agreed that a clinical geneticist usually is responsible for the interpretation of the genetic test results but that the process should be shared between the clinical geneticist, the physician who ordered the test (typically a child neurologist or neuromuscular specialist), and/or a genetic counselor.

Although improving the clarity of genetic test reports and directing physicians to the appropriate specialists for assistance would ultimately ensure a quicker and more accurate genetic diagnosis for patients, it is also important for physicians (nongeneticists) to understand genetic test results, because this will have a direct impact on patient management. The

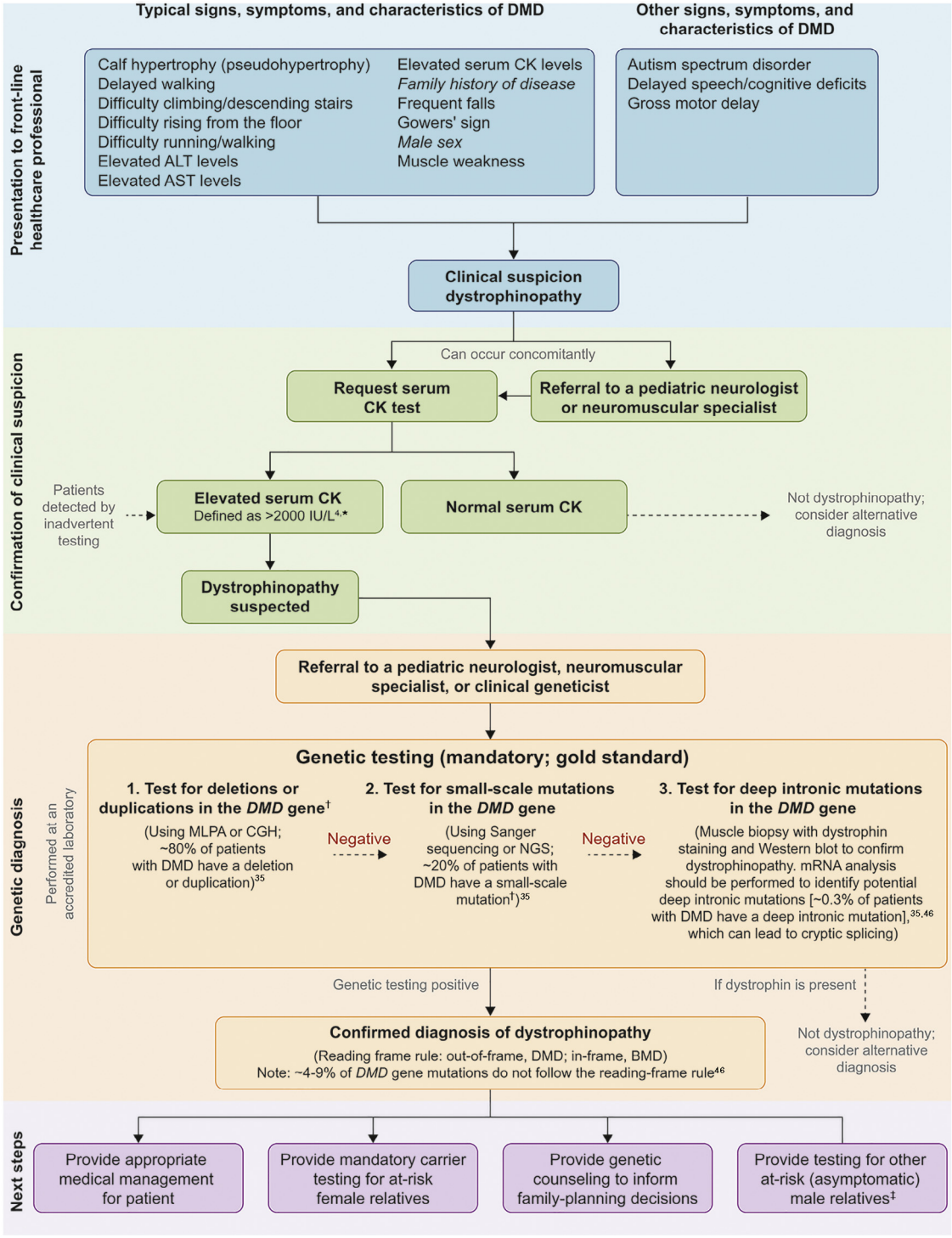

Figure 3. Diagnostic steps for reaching a complete diagnosis of DMD. *ALT*, alanine aminotransferase; *AST*, aspartate aminotransferase; *NGS*, next-generation sequencing. *Elevated CK levels within the range of approximately 500-1200 U/L (1.5 times the upper limit of normal for men), even if asymptomatic, may be indicative of other neuromuscular disorders that require further assessment.[103] †Point mutations or polymorphisms along the probe hybridization region can present as single-exon deletions when using MLPA and thus a second confirmatory test (typically Sanger sequencing) may be required.[11,12,31] ‡Testing of at-risk (asymptomatic) male relatives will be dependent on country-specific legislation.

consensus group therefore agreed that educational meetings for physicians and laboratory specialists on topics relating to the genetic diagnosis of DMD would help to improve physicians' understanding of genetic tests and the interpretation of the results.

The consensus group also agreed on 3 statements to highlight the next steps that should be taken after a complete diagnosis has been reached. These statements highlight the importance of carrier testing, genetic counseling, family-planning decisions, and prenatal diagnosis. It is estimated that approximately one-third of patients with DMD develop the disease owing to de novo mutations and that the remaining two-thirds inherit the mutation from carrier mothers.[11,63,64] If the mutation identified in the affected patient is not carried by the mother in her somatic cell line, the mother may be a germline mosaic and should be provided with counseling about the risk of having a second son with the disease[12] or having a daughter that is a carrier.[11] Female carriers are typically asymptomatic because DMD is an X-linked inherited disease; however, carriers can develop mild clinical symptoms, including muscle weakness and scoliosis,[65,66] and are at an increased risk of cardiomyopathy.[67-69] Despite this, approximately one-third of potential carriers are likely unaware of their carrier status.[64,70] Genetic testing is thus important for family-planning decisions and counseling of other family members.[71,74] Genetic information also is used for genotype–phenotype correlations,[7,75,76,79,114] so that patients can be provided with prognostic information and be offered appropriate medical management.[11,23,77,78]

As per the 2010 best practice guidelines for the molecular diagnosis of DMD/BMD, prenatal testing is advised to be carried out only for at-risk male pregnancies (those with a family history of the disease).[12] Prenatal screening is not currently recommended for female fetuses, because it is not yet possible to determine whether a female heterozygote for a *DMD* mutation will exhibit any signs of disease.[12] However, the consensus group agreed that when a family history is present, prenatal testing leads to earlier detection of DMD and is thus important for family-planning decisions. Recommendations regarding prenatal diagnosis will depend on country-specific legislation.[12,31] The consensus group also agreed that in the presence of a family history of disease, presymptomatic CK testing would lead to earlier detection and thus earlier management of patients with DMD, but would be dependent on country-specific legislation.

The 195th European Neuromuscular Centre International Workshop (2012) report presented discussions on newborn screening (NBS) for DMD; the meeting was attended by 21 experts from 7 countries. It was discussed that the introduction of NBS for DMD using CK testing would help to detect the disease earlier in patients and reduce the risk of having additional children with DMD.[1] A pilot study in the US demonstrated the feasibility of a 2-tier NBS system for DMD using dried blood spots to test CK levels, followed by *DMD* gene testing[96]; however, there are many complexities involved with NBS programs, some of which are discussed in the **Appendix**.

Conclusions

Delays in the diagnosis of DMD have remained relatively unchanged over the last 30 years,[9,10] despite advances in our understanding of the natural history and improvements in genetic testing. Delays occur early in the diagnostic pathway, because of a lack of awareness of DMD and its signs and symptoms among families, and, more pertinently, among front-line healthcare professionals.[4] Issues relating to the understanding of genetic testing required to obtain a complete diagnosis of DMD have been highlighted (**Table I**).[11] Patients presenting with the typical motor signs and symptoms of DMD, as well as the less well-recognized neurocognitive deficits, developmental delays, and elevated liver enzymes, should be immediately referred to a specialist (child neurologist or neuromuscular specialist) and should have their serum CK levels measured. Patients with a marked elevation in serum CK should be referred to a clinical geneticist as soon as possible, and the full range of sequential genetic tests offered to provide a complete diagnosis. After diagnosis, it is mandatory to offer carrier testing to mothers and other at-risk female relatives. By highlighting these issues and providing an in-depth discussion of the DMD diagnostic pathway, we hope that patients will be diagnosed earlier, care provided as soon as possible, and personalized intervention provided for eligible patients. ■

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Disclaimer

The Delphi Consensus statements presented here are based on the opinions of carefully selected experts in the field and are for information and educational purposes only. The statements may reflect gaps in current knowledge, but, where possible, have been supported by relevant literature. These statements do not reflect clinical practice guidelines or legal standards of care and, as such, do not include all potential diagnostic or management steps. The responsible physician, in light of all of the circumstances presented by the individual patient, must determine the appropriate treatment, diagnosis, and management.

Endorsements

Endorsed by the European Paediatric Neurology Society (EPNS), the Muscular Dystrophy Association (MDA), Duchenne Parent Project Italy, Parent Project Muscular Dystrophy (PPMD), and TREAT-NMD.

References

1. Ellis JA, Vroom E, Muntoni F. 195th ENMC International Workshop: newborn screening for Duchenne muscular dystrophy December 14-16, 2012, Naarden, The Netherlands. Neuromuscul Disord 2013;23:682-9.
2. Hoffman EP, Brown RH Jr, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 1987;51:919-28.
3. Birnkrant DJ, Bushby K, Bann CM, Apkon SD, Blackwell A, Brumbaugh D, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. Lancet Neurol 2018;17:251-67.
4. Ciafaloni E, Fox DJ, Pandya S, Westfield CP, Puzhankara S, Romitti PA, et al. Delayed diagnosis in Duchenne muscular dystrophy: data from the Muscular Dystrophy Surveillance, Tracking, and Research Network (MD STARnet). J Pediatr 2009;155:380-5.
5. D'Amico A, Catteruccia M, Baranello G, Politano L, Govoni A, Previtali SC, et al. Diagnosis of Duchenne Muscular Dystrophy in Italy in the last decade: critical issues and areas for improvements. Neuromuscul Disord 2017;27:447-51.
6. Vry J, Gramsch K, Rodger S, Thompson R, Steffensen BF, Rahbek J, et al. European cross-sectional survey of current care practices for Duchenne muscular dystrophy reveals regional and age-dependent differences. J Neuromuscul Dis 2016;3:517-27.
7. Bello L, Morgenroth LP, Gordish-Dressman H, Hoffman EP, McDonald CM, Cirak S, et al. DMD genotypes and loss of ambulation in the CINRG Duchenne natural history study. Neurology 2016;87:401-9.
8. van Ruiten HJ, Straub V, Bushby K, Guglieri M. Improving recognition of Duchenne muscular dystrophy: a retrospective case note review. Arch Dis Child 2014;99:1074-7.
9. Crisp DE, Ziter FA, Bray PF. Diagnostic delay in Duchenne's muscular dystrophy. JAMA 1982;247:478-80.
10. Firth MA. Diagnosis of Duchenne muscular dystrophy: experiences of parents of sufferers. Br Med J (Clin Res Ed) 1983;286:700-1.
11. Aartsma-Rus A, Ginjaar IB, Bushby K. The importance of genetic diagnosis for Duchenne muscular dystrophy. J Med Genet 2016;53:145-51.
12. Abbs S, Tuffery-Giraud S, Bakker E, Ferlini A, Sejersen T, Mueller CR. Best practice guidelines on molecular diagnostics in Duchenne/Becker muscular dystrophies. Neuromuscul Disord 2010;20:422-7.
13. Rosenfeld RM, Nnacheta LC, Corrigan MD. Clinical consensus statement development manual. Otolaryngol Head Neck Surg 2015;153:S1-14.
14. Guyatt GH, Cook DJ, Sackett DL, Eckman M, Pauker S. Grades of recommendation for antithrombotic agents. Chest 1998;114:441S-444S.
15. Guyatt GH, Oxman AD, Kunz R, Falck-Ytter Y, Vist GE, Liberati A, et al. GRADE: going from evidence to recommendations. BMJ 2008;336:1049-51.
16. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 2008;336:924-6.
17. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Schunemann HJ. GRADE: what is "quality of evidence" and why is it important to clinicians? BMJ 2008;336:995-8.
18. Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. Lancet Neurol 2010;9:77-93.
19. Wong SH, McClaren BJ, Archibald AD, Weeks A, Langmaid T, Ryan MM, et al. A mixed methods study of age at diagnosis and diagnostic odyssey for Duchenne muscular dystrophy. Eur J Hum Genet 2015;23:1294-300.
20. Na SJ, Kim WJ, Kim SM, Lee KO, Yoon B, Choi YC. Clinical, immunohistochemical, Western blot, and genetic analysis in dystrophinopathy. J Clin Neurosci 2013;20:1099-105.
21. Dey S, Senapati AK, Pandit A, Biswas A, Guin DS, Joardar A, et al. Genetic and clinical profile of patients of Duchenne muscular dystrophy: experience from a tertiary care center in Eastern India. Indian Pediatr 2015;52:481-4.
22. Holtzer C, Meaney FJ, Andrews J, Ciafaloni E, Fox DJ, James KA, et al. Disparities in the diagnostic process of Duchenne and Becker muscular dystrophy. Genet Med 2011;13:942-7.
23. Magri F, Govoni A, D'Angelo MG, Del Bo R, Ghezzi S, Sandra G, et al. Genotype and phenotype characterization in a large dystrophinopathic cohort with extended follow-up. J Neurol 2011;258:1610-23.
24. Ricotti V, Mandy WP, Scoto M, Pane M, Deconinck N, Messina S, et al. Neurodevelopmental, emotional, and behavioural problems in Duchenne muscular dystrophy in relation to underlying dystrophin gene mutations. Dev Med Child Neurol 2016;58:77-84.
25. Sarrazin E, von der Hagen M, Schara U, von Au K, Kaindl AM. Growth and psychomotor development of patients with Duchenne muscular dystrophy. Eur J Paediatr Neurol 2014;18:38-44.
26. Colombo P, Nobile M, Tesei A, Civati F, Gandossini S, Mani E, et al. Assessing mental health in boys with Duchenne muscular dystrophy: emotional, behavioural and neurodevelopmental profile in an Italian clinical sample. Eur J Paediatr Neurol 2017;21:639-47.
27. Latimer R, Street N, Conway KC, James K, Cunniff C, Oleszek J, et al. Secondary conditions among males with Duchenne or Becker muscular dystrophy. J Child Neurol 2017;32:663-70.
28. Mirski KT, Crawford TO. Motor and cognitive delay in Duchenne muscular dystrophy: implication for early diagnosis. J Pediatr 2014;165:1008-10.
29. Snow WM, Anderson JE, Jakobson LS. Neuropsychological and neurobehavioral functioning in Duchenne muscular dystrophy: a review. Neurosci Biobehav Rev 2013;37:743-52.
30. Vicari S, Piccini G, Mercuri E, Battini R, Chieffo D, Bulgheroni S, et al. Implicit learning deficit in children with Duchenne muscular dystrophy: evidence for a cerebellar cognitive impairment? PLoS ONE 2018;13:e0191164.
31. Araujo APQC, Carvalho AAS, Cavalcanti EBU, Saute JAM, Carvalho E, Franca MCJ, et al. Brazilian consensus on Duchenne muscular dystrophy. Part 1: diagnosis, steroid therapy and perspectives. Arq Neuropsiquiatr 2017;75:104-13.
32. Hughes BP. Serum enzyme changes in muscle disease and their relation to tissue change. Proc R Soc Med 1963;56:179-82.
33. Strehle EM, Straub V. Recent advances in the management of Duchenne muscular dystrophy. Arch Dis Child 2015;100:1173-7.
34. Hegde MR, Chin EL, Mulle JG, Okou DT, Warren ST, Zwick ME. Microarray-based mutation detection in the dystrophin gene. Hum Mutat 2008;29:1091-9.
35. Bladen CL, Salgado D, Monges S, Foncuberta ME, Kekou K, Kosma K, et al. The TREAT-NMD DMD Global Database: analysis of more than 7,000 Duchenne muscular dystrophy mutations. Hum Mutat 2015;36:395-402.
36. Juan-Mateu J, Gonzalez-Quereda L, Rodriguez MJ, Baena M, Verdura E, Nascimento A, et al. DMD mutations in 576 dystrophinopathy families: a step forward in genotype-phenotype correlations. PLoS ONE 2015;10:e0135189.
37. Zhong J, Xu T, Chen G, Liao H, Zhang J, Lan D. Genetic analysis of the dystrophin gene in children with Duchenne and Becker muscular dystrophies. Muscle Nerve 2017;56:117-21.
38. Lalic T, Vossen RH, Coffa J, Schouten JP, Guc-Scekic M, Radivojevic D, et al. Deletion and duplication screening in the DMD gene using MLPA. Eur J Hum Genet 2005;13:1231-4.
39. Manjunath M, Kiran P, Preethish-Kumar V, Nalini A, Singh RJ, Gayathri N. A comparative study of mPCR, MLPA, and muscle biopsy results in a cohort of children with Duchenne muscular dystrophy: a first study. Neurol India 2015;63:58-62.
40. Khordadpoor-Deilamani F, Akbari MT, Nafissi S, Zamani G. Dystrophin gene mutation analysis in Iranian males and females using multiplex polymerase chain reaction and multiplex ligation-dependent probe amplification methods. Genet Test Mol Biomarkers 2011;15:893-9.

41. Dastur RS, Kachwala MY, Khadilkar SV, Hegde MR, Gaitonde PS. Identification of deletions and duplications in the Duchenne muscular dystrophy gene and female carrier status in western India using combined methods of multiplex polymerase chain reaction and multiplex ligation-dependent probe amplification. Neurol India 2011;59:803-9.
42. Bovolenta M, Neri M, Fini S, Fabris M, Trabanelli C, Venturoli A, et al. A novel custom high density-comparative genomic hybridization array detects common rearrangements as well as deep intronic mutations in dystrophinopathies. BMC Genomics 2008;9:572.
43. Baskin B, Gibson WT, Ray PN. Duchenne muscular dystrophy caused by a complex rearrangement between intron 43 of the *DMD* gene and chromosome 4. Neuromuscul Disord 2011;21:178-82.
44. Ishmukhametova A, Khau Van Kien P, Mechin D, Thorel D, Vincent MC, Rivier F, et al. Comprehensive oligonucleotide array-comparative genomic hybridization analysis: new insights into the molecular pathology of the *DMD* gene. Eur J Hum Genet 2012;20:1096-100.
45. Santos R, Goncalves A, Oliveira J, Vieira E, Vieira JP, Evangelista T, et al. New variants, challenges and pitfalls in DMD genotyping: implications in diagnosis, prognosis and therapy. J Hum Genet 2014;59:454-64.
46. Tuffery-Giraud S, Miro J, Koenig M, Claustres M. Normal and altered pre-mRNA processing in the *DMD* gene. Hum Genet 2017;136:1155-72.
47. Roucher Boulez F, Menassa R, Streichenberger N, Manel V, Mallet-Motak D, Morel Y, et al. A splicing mutation in the DMD gene detected by next-generation sequencing and confirmed by mRNA and protein analysis. Clin Chim Acta 2015;448:146-9.
48. Magri F, Del Bo R, D'Angelo MG, Govoni A, Ghezzi S, Gandossini S, et al. Clinical and molecular characterization of a cohort of patients with novel nucleotide alterations of the dystrophin gene detected by direct sequencing. BMC Med Genet 2011;12:37.
49. Niba ETE, Nishida A, Tran VK, Vu DC, Matsumoto M, Awano H, et al. Cryptic splice activation but not exon skipping is observed in minigene assays of dystrophin c.9361 + 1G>A mutation identified by NGS. J Hum Genet 2017;62:531-7.
50. Wang Z, Lin Y, Qiu L, Zheng D, Yan A, Zeng J, et al. Hybrid minigene splicing assay verified the pathogenicity of a novel splice site variant in the dystrophin gene of a Chinese patient with typical Duchenne muscular dystrophy phenotype. Clin Chem Lab Med 2016;54:1435-40.
51. Baskin B, Stavropoulos DJ, Rebeiro PA, Orr J, Li M, Steele L, et al. Complex genomic rearrangements in the dystrophin gene due to replication-based mechanisms. Mol Genet Genomic Med 2014;2:539-47.
52. Khelifi MM, Ishmukhametova A, Khau Van Kien P, Thorel D, Mechin D, Perelman S, et al. Pure intronic rearrangements leading to aberrant pseudoexon inclusion in dystrophinopathy: a new class of mutations? Hum Mutat 2011;32:467-75.
53. Greer K, Mizzi K, Rice E, Kuster L, Barrero RA, Bellgard MI, et al. Pseudoexon activation increases phenotype severity in a Becker muscular dystrophy patient. Mol Genet Genomic Med 2015;3:320-6.
54. Zaum AK, Stuve B, Gehrig A, Kolbel H, Schara U, Kress W, et al. Deep intronic variants introduce DMD pseudoexon in patient with muscular dystrophy. Neuromuscul Disord 2017;27:631-4.
55. Williams MS. Genetics and managed care: policy statement of the American College of Medical Genetics. Genet Med 2001;3:430-5.
56. Baars MJ, Henneman L, Ten Kate LP. Deficiency of knowledge of genetics and genetic tests among general practitioners, gynecologists, and pediatricians: a global problem. Genet Med 2005;7:605-10.
57. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24.
58. Thurston VC, Wales PS, Bell MA, Torbeck L, Brokaw JJ. The current status of medical genetics instruction in US and Canadian medical schools. Acad Med 2007;82:441-5.
59. Greb AE, Brennan S, McParlane L, Page R, Bridge PD. Retention of medical genetics knowledge and skills by medical students. Genet Med 2009;11:365-70.
60. Klitzman R, Chung W, Marder K, Shanmugham A, Chin LJ, Stark M, et al. Attitudes and practices among internists concerning genetic testing. J Genet Couns 2013;22:90-100.
61. Burke S, Stone A, Bedward J, Thomas H, Farndon P. A "neglected part of the curriculum" or "of limited use"? Views on genetics training by nongenetics medical trainees and implications for delivery. Genet Med 2006;8:109-15.
62. Shields AE, Burke W, Levy DE. Differential use of available genetic tests among primary care physicians in the United States: results of a national survey. Genet Med 2008;10:404-14.
63. Lee T, Takeshima Y, Kusunoki N, Awano H, Yagi M, Matsuo M, et al. Differences in carrier frequency between mothers of Duchenne and Becker muscular dystrophy patients. J Hum Genet 2014;59:46-50.
64. Helderman-van den Enden ATJM, van den Bergen JC, Breuning MH, Verschuuren JJGM, Tibben A, Bakker E, et al. Duchenne/Becker muscular dystrophy in the family: have potential carriers been tested at a molecular level? Clin Genet 2011;79:236-42.
65. Juan-Mateu J, Rodriguez MJ, Nascimento A, Jimenez-Mallebrera C, Gonzalez-Quereda L, Rivas E, et al. Prognostic value of X-chromosome inactivation in symptomatic female carriers of dystrophinopathy. Orphanet J Rare Dis 2012;7:82.
66. Papa R, Madia F, Bartolomeo D, Trucco F, Pedemonte M, Traverso M, et al. Genetic and early clinical manifestations of females heterozygous for Duchenne/Becker muscular dystrophy. Pediatr Neurol 2016;55:58-63.
67. McCaffrey T, Guglieri M, Murphy AP, Bushby K, Johnson A, Bourke JP. Cardiac involvement in female carriers of Duchenne or Becker muscular dystrophy. Muscle Nerve 2017;55:810-8.
68. Schelhorn J, Schoenecker A, Neudorf U, Schemuth H, Nensa F, Nassenstein K, et al. Cardiac pathologies in female carriers of Duchenne muscular dystrophy assessed by cardiovascular magnetic resonance imaging. Eur Radiol 2015;25:3066-72.
69. Florian A, Rosch S, Bietenbeck M, Engelen M, Stypmann J, Waltenberger J, et al. Cardiac involvement in female Duchenne and Becker muscular dystrophy carriers in comparison to their first-degree male relatives: a comparative cardiovascular magnetic resonance study. Eur Heart J Cardiovasc Imaging 2016;17:326-33.
70. Bogue L, Peay H, Martin A, Lucas A, Ramchandren S. Knowledge of carrier status and barriers to testing among mothers of sons with Duchenne or Becker muscular dystrophy. Neuromuscul Disord 2016;26:860-4.
71. Plumridge G, Metcalfe A, Coad J, Gill P. Parents' communication with siblings of children affected by an inherited genetic condition. J Genet Couns 2011;20:374-83.
72. Hoffman EP, Arahata K, Minetti C, Bonilla E, Rowland LP. Dystrophinopathy in isolated cases of myopathy in females. Neurology 1992;42:967-75.
73. Bogue L, Ramchandren S. Outdated risk assessment in a family with Duchenne dystrophy: implications for duty to reassess. Neurol Genet 2016;2:e103.
74. Lehmann A, Speight BS, Kerzin-Storrar L. Extended family impact of genetic testing: the experiences of X-linked carrier grandmothers. J Genet Couns 2011;20:365-73.
75. Hightower RM, Alexander MS. Genetic modifiers of Duchenne and facioscapulohumeral muscular dystrophies. Muscle Nerve 2018;57:6-15.
76. Pons R, Kekou K, Gkika A, Papadimas G, Vogiatzakis N, Svingou M, et al. Single amino acid loss in the dystrophin protein associated with a mild clinical phenotype. Muscle Nerve 2017;55:46-50.
77. Taylor PJ, Betts GA, Maroulis S, Gilissen C, Pedersen RL, Mowat DR, et al. Dystrophin gene mutation location and the risk of cognitive impairment in Duchenne muscular dystrophy. PLoS ONE 2010;5:e8803.
78. Ricotti V, Jagle H, Theodorou M, Moore AT, Muntoni F, Thompson DA. Ocular and neurodevelopmental features of Duchenne muscular

dystrophy: a signature of dystrophin function in the central nervous system. Eur J Hum Genet 2016;24:562-8.
79. van den Bergen JC, Ginjaar HB, Niks EH, Aartsma-Rus A, Verschuuren JJ. Prolonged ambulation in Duchenne patients with a mutation amenable to exon 44 skipping. J Neuromuscul Dis 2014;1:91-4.
80. Barp A, Bello L, Politano L, Melacini P, Calore C, Polo A, et al. Genetic modifiers of Duchenne muscular dystrophy and dilated cardiomyopathy. PLoS ONE 2015;10:e0141240.
81. Imbornoni L, Price ET, Andrews J, Meaney FJ, Ciafaloni E, Cunniff C. Diagnostic and clinical characteristics of early-manifesting females with Duchenne or Becker muscular dystrophy. Am J Med Genet A 2014;164A:2769-74.
82. Mercier S, Toutain A, Toussaint A, Raynaud M, de Barace C, Marcorelles P, et al. Genetic and clinical specificity of 26 symptomatic carriers for dystrophinopathies at pediatric age. Eur J Hum Genet 2013;21:855-63.
83. Lang SM, Shugh S, Mazur W, Sticka JJ, Rattan MS, Jefferies JL, et al. Myocardial fibrosis and left ventricular dysfunction in Duchenne muscular dystrophy carriers using cardiac magnetic resonance imaging. Pediatr Cardiol 2015;36:1495-501.
84. Nozoe KT, Akamine RT, Mazzotti DR, Polesel DN, Grossklauss LF, Tufik S, et al. Phenotypic contrasts of Duchenne muscular dystrophy in women: two case reports. Sleep Sci 2016;9:129-33.
85. Parent JJ, Moore RA, Taylor MD, Towbin JA, Jefferies JL. Left ventricular noncompaction cardiomyopathy in Duchenne muscular dystrophy carriers. J Cardiol Cases 2015;11:7-9.
86. Cheng VE, Prior DL. Peripartum cardiomyopathy in a previously asymptomatic carrier of Duchenne muscular dystrophy. Heart Lung Circ 2013;22:677-81.
87. Martinez HR, Pignatelli R, Belmont JW, Craigen WJ, Jefferies JL. Childhood onset of left ventricular dysfunction in a female manifesting carrier of muscular dystrophy. Am J Med Genet A 2011;155A:3025-9.
88. Yilmaz A, Gdynia HJ, Ludolph AC, Klingel K, Kandolf R, Sechtem U. Images in cardiovascular medicine. Cardiomyopathy in a Duchenne muscular dystrophy carrier and her diseased son: similar pattern revealed by cardiovascular MRI. Circulation 2010;121:e237-9.
89. Walcher T, Kunze M, Steinbach P, Sperfeld AD, Burgstahler C, Hombach V, et al. Cardiac involvement in a female carrier of Duchenne muscular dystrophy. Int J Cardiol 2010;138:302-5.
90. McGowan R, Challoner BR, Ross S, Holloway S, Joss S, Wilcox D, et al. Results of Duchenne muscular dystrophy family screening in practice: leaks rather than cascades? Clin Genet 2013;83:187-90.
91. Bermudez-Lopez C, Garcia-de Teresa B, Gonzalez-del Angel A, Alcantara-Ortigoza MA. Germinal mosaicism in a sample of families with Duchenne/Becker muscular dystrophy with partial deletions in the *DMD* gene. Genet Test Mol Biomarkers 2014;18:93-7.
92. Garcia S, de Haro T, Zafra-Ceres M, Poyatos A, Gomez-Capilla JA, Gomez-Llorente C. Identification of *de novo* mutations of Duchenne/Becker muscular dystrophies in southern Spain. Int J Med Sci 2014;11:988-93.
93. Strmecki L, Hudler P, Benedik-Dolnicar M, Komel R. De novo mutation in DMD gene in a patient with combined hemophilia A and Duchenne muscular dystrophy. Int J Hematol 2014;99:184-7.
94. Luna-Angulo AB, Gomez-Diaz B, Escobar-Cedillo RE, Anaya-Segura MA, Estrada-Mena FJ, Lopez-Hernandez LB. A new de novo mutation in a non-hot spot region at the DMD gene in a Mexican family. Genet Couns 2014;25:429-32.
95. Li T, Zhang ZJ, Ma X, Lv X, Xiao H, Guo QN, et al. Prenatal diagnosis for a Chinese family with a de novo *DMD* gene mutation: a case report. Medicine (Baltimore) 2017;96:e8814.
96. Mendell JR, Shilling C, Leslie ND, Flanigan KM, al-Dahhak R, Gastier-Foster J, et al. Evidence-based path to newborn screening for Duchenne muscular dystrophy. Ann Neurol 2012;71:304-13.
97. Moat SJ, Bradley DM, Salmon R, Clarke A, Hartley L. Newborn bloodspot screening for Duchenne muscular dystrophy: 21 years experience in Wales (UK). Eur J Hum Genet 2013;21:1049-53.
98. Ke Q, Zhao ZY, Griggs R, Wiley V, Connolly A, Kwon J, et al. Newborn screening for Duchenne muscular dystrophy in China: follow-up diagnosis and subsequent treatment. World J Pediatr 2017;13:197-201.
99. Wood MF, Hughes SC, Hache LP, Naylor EW, Abdel-Hamid HZ, Barmada MM, et al. Parental attitudes toward newborn screening for Duchenne/Becker muscular dystrophy and spinal muscular atrophy. Muscle Nerve 2014;49:822-8.
100. Lillie SE, Tarini BA, Janz NK, Zikmund-Fisher BJ. Framing optional genetic testing in the context of mandatory newborn screening tests. BMC Med Inform Decis Mak 2015;15:50.
101. Helderman-van den Enden AT, Madan K, Breuning MH, van der Hout AH, Bakker E, de Die-Smulders CE, et al. An urgent need for a change in policy revealed by a study on prenatal testing for Duchenne muscular dystrophy. Eur J Hum Genet 2013;21:21-6.
102. Wang H, Xu Y, Liu X, Wang L, Jiang W, Xiao B, et al. Prenatal diagnosis of Duchenne muscular dystrophy in 131 Chinese families with dystrophinopathy. Prenat Diagn 2017;37:356-64.
103. Moghadam-Kia S, Oddis CV, Aggarwal R. Approach to asymptomatic creatine kinase elevation. Cleve Clin J Med 2016;83:37-42.
104. The European Bioinformatics Institute (EMBL-EBI). EBI Search: gene & protein summary for dystrophin. https://www.ebi.ac.uk/s4/summary/molecular/gene?term=dystrophin&classification=9606&tid=protOrthDMDHUMAN. Accessed November 26, 2018.
105. Grimm T, Kress W, Meng G, Muller CR. Risk assessment and genetic counseling in families with Duchenne muscular dystrophy. Acta Myol 2012;31:179-83.
106. Takeshima Y, Yagi M, Okizuka Y, Awano H, Zhang Z, Yamauchi Y, et al. Mutation spectrum of the dystrophin gene in 442 Duchenne/Becker muscular dystrophy cases from one Japanese referral center. J Hum Genet 2010;55:379-88.
107. Rani AQ, Sasongko TH, Sulong S, Bunyan D, Salmi AR, Zilfalil BA, et al. Mutation spectrum of dystrophin gene in Malaysian patients with Duchenne/Becker muscular dystrophy. J Neurogenet 2013;27:11-5.
108. Zimowski JG, Massalska D, Holding M, Jadczak S, Fidzianska E, Lusakowska A, et al. MLPA based detection of mutations in the dystrophin gene of 180 Polish families with Duchenne/Becker muscular dystrophy. Neurol Neurochir Pol 2014;48:416-22.
109. de Almeida PA, Machado-Costa MC, Manzoli GN, Ferreira LS, Rodrigues MC, Bueno LS, et al. Genetic profile of Brazilian patients with dystrophinopathies. Clin Genet 2017;92:199-203.
110. Ramos E, Conde JG, Berrios RA, Pardo S, Gomez O, Mas Rodriguez MF. Prevalence and genetic profile of Duchene and Becker muscular dystrophy in Puerto Rico. J Neuromuscul Dis 2016;3:261-6.
111. Griffiths AJF, Gelbart WM, Miller JH, Lewontin RC. Chromosomal rearrangements. In: Modern genetic analysis. 1999 https://www.ncbi.nlm.nih.gov/books/NBK21367/. Accessed November 26, 2018.
112. Wang Y, Yang Y, Liu J, Chen XC, Liu X, Wang CZ, et al. Whole dystrophin gene analysis by next-generation sequencing: a comprehensive genetic diagnosis of Duchenne and Becker muscular dystrophy. Mol Genet Genomics 2014;289:1013-21.
113. Cummings BB, Marshall JL, Tukiainen T, Lek M, Donkervoort S, Foley AR, et al. Improving genetic diagnosis in Mendelian disease with transcriptome sequencing. Sci Transl Med 2017;9:eaal5209.
114. Bello L, Kesari A, Gordish-Dressman H, Cnaan A, Morgenroth LP, Punetha J, et al. Genetic modifiers of ambulation in the Cooperative International Neuromuscular Research Group Duchenne natural history study. Ann Neurol 2015;77:684-96.

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Appendix

Introduction

Example survey results from the 2017 and 2018 TREAT-NMD Expert Masterclasses regarding the genetic diagnosis of patients with DMD are presented in **Table I**, available at www.jpeds.com.

Methods

Systematic Literature Searches. Search terms were identified by the steering committee and literature searches performed in Ovid (2017 Ovid Technologies, Inc, New York, New York) by PharmaGenesis London, London, United Kingdom, to screen the MEDLINE and EMBASE databases. Search terms (title/abstract) were: *Duchenne muscular dystrophy*, *DMD* or *Duchenne* AND any of the following search terms: *sign*; *symptom*; *creatine kinase*; *transaminase*; *diagnos**; *gene**; *genetic test**; *genetic report**; *genetic counsel**; *delay**; *screen**; *carrier*; *mutation*; *sequenc**; *comparative genome hybridi?ation*; *CGH*; *multiplex ligation-dependent probe amplification*; *MLPA*; *multiplex polymerase chain reaction*; *multiplex PCR*; *biops**; *prenatal*; *neonatal*; *female*; *family planning*; *germline mosaicism*; *de novo*; *practitioner*; *p?ediatrician*; *p?ediatric neurologist*; *neuromuscular specialist*. The "?" and "*" functions searched for spelling variations and variations of the word ending, respectively. Search results were limited to: articles published from January 1, 2010, to April 8, 2018; English; studies of humans; and full journal articles. The full electronic search strategy is provided in **Table II**, available at www.jpeds.com. PharmaGenesis London exported the initial raw results and highlighted articles of potential relevance using a traffic light system (red—unlikely to be relevant; amber—potentially relevant; green—likely to be relevant). The 3 members of the steering committee then reviewed the relevance of the supporting literature. Any articles included by the authors that were not identified by the systematic literature review were categorized as ad hoc in the PRISMA flow diagram in **Figure 2**, available at www.jpeds.com. Data were then extracted by PharmaGenesis London, and used, under the guidance of the steering committee, to draft questions to aid the development of the Consensus Statements.

Development of the Consensus Statements via Iterative Voting. Voting round 1 included 29 questions drafted by the steering committee based on initial review of the literature, to which the consensus group provided free-text responses. The results were collated by PharmaGenesis London to ensure voter anonymity, and the anonymized results were reviewed by the nonvoting chair. During each voting round, the consensus group was encouraged to comment on the wording and content of the questions, themes, and statements. After each round, the steering committee made changes to the statements to reflect the feedback received.

Voting round 2 included 20 questions formulated using the responses from round 1. The consensus group then rated the importance of, or agreement with, the themes identified during round 2 (using a ranking or 5-point Likert scale). Voting rounds 1 and 2 were conducted using SurveyMonkey (https://www.surveymonkey.co.uk/).

Voting round 3 was developed in advance of a 1-day consensus meeting held in May 2017 in Copenhagen, Denmark, and the draft statements were circulated to the consensus group before the meeting. This round comprised 24 statements for which the consensus group had to rate their level of agreement using a 5-point Likert scale ("strongly agree" [A+]; "agree" [A]; "neither agree nor disagree" [N]; "disagree" [D]; or "strongly disagree" [D+]; **Figure 1**.[1-4]) using anonymized electronic keypads. Consensus was defined a priori as at least 75% agreement (either "strongly agree" [A+] or "agree" [A]). Each question was introduced by the nonvoting chair. If consensus was not reached after the first round of voting, alterations to the statement were made based on discussions before the next round of voting was conducted.

The 3 members of the steering committee then graded the level of evidence and strength of recommendations supporting each statement using the Grading of Recommendations Assessment, Development, and Evaluation system (**Figure 1**). The grading was reviewed and agreed on by the expert voting panel (**Table III**).

Additional Supporting Information for the Consensus Statements. Additional supporting information from the systematic literature review for each consensus statement is detailed below.

Section 1: Reducing the Time to Diagnosis of DMD (Table III).

Statement 1. The following signs, symptoms, and characteristics should be considered typical indicators of DMD: calf hypertrophy (pseudohypertrophy); delayed walking; difficulty climbing/descending stairs; difficulty rising from the floor; difficulty running/walking; elevated serum CK level (including elevated alanine transaminase and aspartate transaminase); a family history of DMD; frequent falls; Gowers' sign; male sex; and muscle weakness.

Vote: A+ = 93%; A = 7%; grade of recommendation: 1B

Discussion

In support of this statement (**Table III**), a retrospective chart review of 156 boys with DMD (no family history of disease) using the Muscular Dystrophy Surveillance, Tracking, and Research Network (MD STARnet) assessed the range of signs and symptoms first reported to healthcare providers by patients who were later diagnosed with DMD (n = 111).[5] In patients aged 3 to <5 years, the signs and symptoms included calf hypertrophy, 7.1%; difficulty rising from the floor, 23.8%; difficulty climbing, 31.0%; frequent falls/clumsiness, 33.3%; difficulty running/walking, 38.1%; and muscle weakness, 40.5%.[5] These symptoms, with the exception of muscle weakness, difficulty running/walking, and difficulty climbing, were the most frequently reported within this age range compared

with younger or older patients with DMD.[5] Muscle weakness and difficulty climbing were reported more frequently in older patients (≥5 years old), and difficulty running/walking was more frequently reported in patients aged 1.5 to <3 years and patients aged ≥5 years, as the symptom(s) first reported to healthcare providers.[5]

These findings are supported by several other studies. First, a mixed-methods study of parents' and patients' experiences of the diagnosis of DMD in Australia reported that the initial symptoms noted by parents (n = 62) (by >20% of parents) were calf hypertrophy, 42%; complaining of tired legs, 26%; difficulties with walking, 35%; frequent falls, 44%; tiring easily, 37%; difficulties running, 65%; and difficulties climbing stairs, 61%.[6] Similarly, a case note review of 20 boys with DMD in the United Kingdom showed that 20% (8/20) of boys reported "difficulty with steps," and 20% (8/20) of boys experienced "falls" as part of their initial symptom profile. Three of the 20 boys experienced delayed first walking (later than the 18-month milestone), and 4 of the boys were diagnosed from an incidental finding of elevated serum CK level.[7] A retrospective review of 24 patients with DMD in Korea reported that their initial symptoms were: difficulty rising from the floor, 16.7%; lower-extremity muscle weakness, 90.0%; family history of DMD, 29.2%; and calf hypertrophy, 87.5%. Mean serum CK levels also were elevated (14 144 IU/L).[8] In addition, a study of patients with DMD from eastern India showed that of the 81 patients assessed, 100% had lower-limb weakness, 97.5% had neck-muscle weakness, 93.8% had calf hypertrophy, 70.4% exhibited Gowers' sign, and 27.1% had a family history of DMD.[9] Lastly, a retrospective study of medical records for 540 patients in the US with DMD showed that patients with a family history of disease are typically seen and diagnosed at an earlier age than those without a family history (with family history vs without family history: age at initial evaluation, 30.8 months vs 56.8 months; $P < .001$; age at CK measurement, 35.1 months vs 64.0 months; $P < .001$).[10] Age at genetic testing did not differ significantly between the 2 groups (56.2 months vs 64.3 months, respectively).

Commentary

The consensus group agreed that patients were sometimes identified inadvertently as a result of high serum CK levels, detected as part of routine screening or assessments unrelated to a diagnosis of DMD. It also was discussed that a family history of DMD would act as a strong indicator of DMD, particularly in a male patient presenting with signs and symptoms of the disease.

Statement 2. Autism spectrum disorder, delayed speech/cognitive deficits, and gross motor delay can be indicators of DMD but are not always associated with the disease.

Vote: A+ = 93%; A = 7%; grade of recommendation: 1B

Discussion

In support of this statement, a European study of 4 centers found that 26% (34/130) of patients with DMD were reported to have an intellectual disability. Of 87 patients who completed the full neurodevelopmental assessment, 21% (18/87) scored in the autistic spectrum disorder range, 24% (21/87) showed clinical hyperactivity, and 44% (38/87) had severe difficulties with inattention.[11] Similarly, a study in Germany of 263 patients with DMD showed that 30% of patients experienced a delay in gross motor development and that approximately 40% of patients showed cognitive impairment (learning difficulty, 26%; intellectual disability, 17%).[12] A smaller case note review of 20 boys with DMD (without a family history of disease) in the United Kingdom showed that speech delay was a presenting feature in 25% of the cohort and that this was identified in 45% of the patients at the time of diagnosis.[7] A study of patients with DMD from eastern India showed that approximately one-third of boys with DMD had mild intellectual disability (IQ 38-63).[9] A recent study of 209 caregivers of boys with DMD identified through MD STARnet showed that cognitive deficits were reported in 38.4% of boys.[13] Furthermore, a chart review study of 179 boys with DMD (1989-2012) showed that delayed walking and cognitive impairment were correlated ($P \leq .0001$).[14] It also has been shown that boys with DMD have a reduced rate of implicit learning compared with boys with typical development, even in the absence of global intellectual disability.[15]

The location of the *DMD* gene mutation also has been shown to correlate with the severity of cognitive impairment. For example, an observational study of 47 Italian boys with DMD showed that Full-Scale IQ scores correlated with the location of the dystrophin gene mutation; mutations in the distal region of the *DMD* gene were associated with more severe cognitive deficits.[16] Patients with point mutations in the *DMD* gene exhibited a higher degree of cognitive impairment than those with deletions or duplications ($P = .005$). In addition, patients with mutations in the distal region of the *DMD* gene had lower IQ levels than those who had mutations in the proximal region.[17] Additional information is presented in a recent review of the literature examining neuropsychological and neurobehavioral functioning in patients with DMD.[18]

These neurocognitive deficits and developmental delays can sometimes be overlooked; as a result, patients with DMD can be referred to the incorrect specialist. This was exemplified in a retrospective chart review of 156 boys with DMD using MD STARnet, which showed that although 16.5% (21/127) of patients were correctly referred to either a neurologist or a neuromuscular specialist, 15.7% (20/127) were referred to a physical, occupational, or speech therapist as a result of their first evaluation.[5]

Commentary

No additional supporting information is included.

Statement 3. After initial presentation, patients with suspected DMD should have their serum CK tested and be referred to a specialist (a pediatric neurologist or neuromuscular specialist).

Vote: A+ = 93%; A = 7%; grade of recommendation: 1B

Discussion

Serum CK level was first recognized as a marker of muscular dystrophy approximately 60 years ago.[19] Levels of serum CK are elevated in patients with muscular dystrophy; this is caused by leakage of cytoplasmic CK from damaged muscle fiber cells into the blood circulation.[20] Findings from a retrospective case note review of 20 boys with DMD (with a family history of the disease) in the United Kingdom showed that a delay in requesting a serum CK level test accounted for the majority of the delay experienced by patients (mean [range] diagnostic delay from first visit with a healthcare professional to diagnosis of DMD, 8.8 [0-50] months; delay in CK level test, 7.2 [0-49] months; and time from CK test result available to genetic diagnosis, 1.6 [0-4] months).[7] The mean (range) age at diagnosis for these patients was 51.7 (10-91) months; however, in 4 boys who had been diagnosed based on incidental serum CK level findings as part of an unrelated illness, the age at diagnosis was greatly reduced (35.3 [10-57] months).[7]

Commentary

The consensus group agreed that a serum CK level test plays a pivotal role in the diagnostic process for DMD and that this should be requested immediately or as soon as possible by primary care physicians, along with referral to a specialist (a pediatric neurologist or a neuromuscular specialist), for any patient presenting with associated signs and symptoms of the disease. Overall, 87% of consensus group members (13/15) indicated that the request for a serum CK level test should be made "immediately" or "as soon as possible" after the patient presents with initial symptoms; the remaining 13% (2/15) indicated that this should be done within 1 week. Similarly, the majority (75% [9/12]) of consensus group members also agreed that referral to a specialist should occur immediately or as soon as possible after receiving a positive CK test result; the remaining 25% (3/12) indicated that this should occur within 1 week.

Statement 4. A lack of awareness of DMD and associated symptoms by the healthcare professional and long waiting times to see a specialist are the primary factors leading to a delay in initial diagnosis.

Vote: A+ = 67%; A = 33%; grade of recommendation: 1C

Discussion

In support of this statement, a retrospective chart review of 156 patients without a family history of DMD using MD STARnet showed that there is a delay of approximately 1 year from first evaluation by the healthcare professional to referral to a neurologist or neuromuscular specialist (mean age of patient ± SD [range]: first evaluation, 3.6 ± 1.7 [0.2-8.0] years; first neurology/neuromuscular specialist visit, 4.6 ± 1.7 [0.3-8.6] years).[5] This highlights a significant delay in the time from first assessment to seeing a specialist. Several patients also were referred incorrectly (eg, to a speech therapist),[5] demonstrating a lack of awareness of some of the signs and symptoms associated with DMD among front-line healthcare professionals. Similarly, a retrospective case note review of 20 boys with DMD (with a family history of the disease) in the United Kingdom showed that in 19 of the boys, the serum CK level test was performed in secondary care, thus delaying the time to diagnosis (10-50 months, n = 15; <10 months, n = 4). The 1 boy whose serum CK level test was requested by a general practitioner had a minimal delay in the time to diagnosis (<10 months),[7] highlighting the importance of recognizing symptoms early. Furthermore, an Australian mixed-methods study has shown that parents visit a range of healthcare professionals in their search for a diagnosis, and 51% of the parents surveyed (29/57) felt that their child could have been diagnosed earlier.[6] Findings from the largest cross-sectional survey of European patients with DMD[21] also showed a delay of approximately 1 year from report of first symptoms to diagnosis (mean delay ± SD: 1.3 ± 1.8 years); however, this varied by country.

Commentary

When asked for their opinion, most consensus group members (93% [13/14]) agreed that a "wait-and-see" approach by healthcare professionals can lead to a delay in the initial diagnosis of DMD. When asked if a "wait-and-see" approach by the family also could lead to a delay in the initial diagnosis, the responses were more varied: 50% answered "yes"; 43% answered "not sure"; and 7% answered "no." An initial delay of approximately 1 year from when the first signs or symptoms are noted in the child (usually by the family, a caregiver, or a school teacher) to the child's first evaluation by a healthcare provider also has been reported.[5] Furthermore, a mixed-methods study of parents' and patients' experiences reported that factors affecting a parent's decision to seek medical help include lack of self-confidence; being a first-time parent; reassurance from others; and the broad range of normal development seen in children.[6] These findings highlight that awareness of DMD needs to be improved generally (not just for front-line healthcare professionals); however, this is beyond the scope of this initiative.

During the meeting, the consensus group members also were asked whether a 4-week wait to see a specialist for additional testing (ie, after an initial clinical diagnosis) was appropriate. Of the 15 consensus group members who responded, 87% (13/15) answered "yes", and 13% (2/15) answered "no." The consensus group members were then asked if their patients are seen within 4 weeks for this additional testing. Of the 15 who responded, 47% (7/15) answered "not always"; 27% (4/15) answered "unsure"; 20% (3/15) answered "yes"; and 7% (1/15) answered "no," demonstrating that delays in seeing a specialist are experienced.

Statement 5. Genetic testing is crucial for obtaining a complete diagnosis of DMD and should be considered the gold standard.

Vote: A+ = 94%; A = 6%; grade of recommendation: 1C

Discussion

Full characterization of the mutation affecting the *DMD* gene is required to determine its predicted effect on the

reading frame. This is important because mutations that cause a frame-shift, or that change an amino acid codon into a stop codon, generally result in a more severe phenotypic presentation of the disease (ie, DMD), owing to the premature termination of translation. This results in truncated, nonfunctional dystrophin protein.[22] In contrast, mutations that do not disrupt the reading frame typically result in a less severe form of the disease (ie, BMD), owing to the production of a shorter but partly functional version of the dystrophin protein.[22]

At this time, there are 2 mutation-specific therapies with a form of approval. Ataluren has received conditional approval from the European Medicines Agency for the treatment of ambulatory patients with nonsense mutation DMD.[23] Eteplirsen has received accelerated approval from the US Food and Drug Administration for the treatment of patients with DMD who have a mutation in the *DMD* gene amenable to mRNA reframing by exclusion of exon 51 from mature mRNA.[24,25]

Commentary

No additional supporting information is included.

Statement 6. In the majority of cases, a complete genetic diagnosis can be made using MLPA or comparative genome hybridization (CGH) to detect deletions or duplications in the *DMD* gene.

Vote: A+ = 88%; A = 6%, N = 6%; grade of recommendation: 1B

Discussion

There are several databases that can be accessed to obtain information on *DMD* gene mutations:

- The Leiden Open Variation Database is an online open source that currently contains more than 10 000 *DMD* gene mutations (www.dmd.nl).[26]
- The UMD-TREAT-NMD database was recently set up as part of an international effort to provide up-to-date information about *DMD* gene mutations worldwide (http://umd.be/TREAT_DMD/); data from more than 30 national registries are collated here.[27]
- The Human Gene Mutation Database (HGMD) is a database for human inherited diseases and is maintained by the Institute of Medical Genetics in Cardiff, United Kingdom (http://www.hgmd.cf.ac.uk/ac/index).[28]

Owing to the fact that approximately 80% of all *DMD* mutations are either large deletions or duplications, it is practical to screen for these mutations as the first step toward reaching a complete genetic diagnosis of DMD.[22] Multiplex polymerase chain reaction (PCR) using the Chamberlain and colleagues, and Beggs and colleagues primer sets, along with more recent primer sets, can be used to detect approximately 98% of all *DMD* gene deletions; however, these assays do not characterize the boundaries of these mutations, do not screen all 79 exons of the *DMD* gene, and are unable to detect duplications.[22,29] MLPA and CGH are quantitative assays and can characterize all of the mutations not detected by multiplex PCR; these tests can also be used for carrier testing in females.[22,29]

Multiplex Ligation-Dependent Probe Amplification. Lalic et al[30] designed and validated the MLPA assay to screen all 79 exons of the *DMD* gene for deletions and duplications. The MLPA assay was then used to test samples from 123 unrelated patients with DMD or BMD who had already been screened by multiplex PCR. The study showed that MLPA was able to detect all previously identified deletions, as well as a number of new mutations that were previously not detected (new deletions, n = 7; new duplications, n = 9; new point mutation, n = 1 [note: point mutations are detected by MLPA only when the mutation lies within the binding site of the MLPA probe]).[30] MLPA also was able to determine the precise genetic rearrangement, which is important for determining the effect on the reading frame.[30]

Ultimately, the study showed that MLPA outperformed the Beggs and Chamberlain multiplex PCR test (detecting ~13% more mutations). In light of this, the authors recommended that MLPA be considered the method of choice for initial DNA analysis of patients with suspected DMD or BMD.[30] The improved detection rate of MLPA compared with multiplex PCR is supported by a number of other studies.[31-33] However, the MLPA assay has a number of limitations: it is unable to provide information regarding the location of intronic breakpoints[22,34] and point mutations or nonpathogenic polymorphisms in the probe binding site can present as single-exon deletions when using this method.[29,35,36] A second confirmatory test, generally Sanger sequencing of the involved exons, should therefore be performed for these false-positive test results.[22,29,34,37] In addition, false-negative test results may occur if there is a partial exonic deletion that is not coincident with the ligation site of the MLPA probe.[36] Despite this, MLPA is a commercially available test and is currently the most widely used.[29]

Comparative Genome Hybridization. Soon after the development of MLPA, a novel DMD-CGH array covering the full genomic region of the *DMD* gene was tested.[38] The assay was able to detect all previously identified deletions and duplications (4/4) and was able to provide the location of intronic breakpoints for these patients. The CGH assay also was able to identify the causative mutations in 3 of 8 of the patients with DMD who had previously tested negative by MLPA.[38] CGH provides a high-resolution assay that has enabled the detection of complex genomic rearrangements and intronic breakpoints[29,39,40] and therefore has a slightly greater mutation detection rate than MLPA. This is supported by several other studies.[33,41]

Ultimately, if deletion/duplication testing is positive, and the mutation is fully characterized and correlates with the severity of symptoms (eg, DMD or BMD), then no further genetic testing is required.[42,43] In some cases, patients with a BMD phenotype have been found to have an out-of-frame *DMD* gene deletion according to MLPA analysis. An explanation for this discrepancy is that with MLPA, only a portion of the exon is

analyzed, and there have been some rare cases reported where the deletion extends to the splice site of the adjacent exon but not the MLPA target site.

Commentary

In the near future, newer assays such as next-generation sequencing (NGS) will be able to evaluate deletions, duplications, and point mutations in a single assay.[44,45] However, because routine NGS is not yet widely available, it was not included as part of this consensus statement.

Statement 7. If exon-level deletions/duplications in the *DMD* gene are not identified, small-scale mutations (by sequencing of exons and flanking regions) should be tested for as the next step.

Vote: A+ = 93%; A = 7%; grade of recommendation: 1C

Discussion

If deletion/duplication testing is negative, it is possible that the patient may have a small-scale mutation in the *DMD* gene (these account for ~20% of all *DMD* gene mutations).[22,46] Despite these findings, a recent survey of 41 individuals (primarily pediatric neurologists and clinical geneticists) from Europe, Turkey, and India at the 2015 TREAT-NMD Expert Masterclass on DMD showed that >10% of respondents did not perform additional tests if deletion/duplication testing was negative.[22] The reasons for this included the cost of Sanger sequencing and the need for shipping of samples to other laboratories for analysis.[22] This was further supported by survey results from the 2017 and 2018 TREAT-NMD Expert Masterclasses on DMD (**Table I**, available at www.jpeds.com), in which 18.8% and 11.1% of the delegates, respectively, did not know the correct next steps following a negative MLPA test result.

Small-scale mutations (single-nucleotide variants) typically are identified using Sanger sequencing of all individual exons[22]; however, because it is becoming more cost- and time-effective,[47] it is likely that in the near future, this methodology will be replaced by next-generation whole-exome sequencing.[22,44,45,48-50]

Commentary

No additional supporting information is included.

Statement 8. Muscle biopsies with dystrophin staining are generally not needed to obtain a complete diagnosis of DMD, unless DNA testing is negative.

Vote: A+ = 73%; A = 27%; grade of recommendation: 1C

Discussion

A deep intronic mutation can cause part of an intron to be incorporated into the dystrophin mRNA if it is recognized as an exon by the splice-site machinery (such sequences are known as cryptic or pseudo-exons). The inclusion of intronic sequences can disrupt the reading frame and generate stop codons.[22] Intronic mutations detected by NGS have been reported in a number of cases of DMD,[51-54] and muscle biopsies also have been used to confirm the presence of aberrant dystrophin mRNA in patients with DMD.[36,39,55-57] In addition to cases of deep intronic mutations, muscle biopsies may also be considered if the patient presents with a discordant phenotype (ie, the genotype would predict DMD, but the patient presents with BMD, or vice versa)[22,58,59]; however, obtaining this information will not ultimately change the disease course or how the patient is managed.[22]

Commentary

For novel or ultra-rare mutations not previously associated with dystrophinopathy, family-based segregation analysis should be recommended (it should be noted that depending on the diagnostic strategies in different countries, segregation analysis is systematically performed).

Statement 9. Delays in the initial clinical diagnosis/referral to a specialist, the sequential nature of the genetic testing process, and incomplete or nonexhaustive genetic testing should be addressed to prevent delays in reaching a complete genetic diagnosis for patients with DMD.

Vote: A+ = 93%; A = 7%; grade of recommendation: 1C

Discussion

Despite improvements in genetic testing, the sequential nature of the genetic testing process has the potential to lead to delays in diagnosis, particularly for patients with rare mutations (ie, those who will be diagnosed at the very end of the diagnostic pathway). However, the availability of NGS will ultimately provide a single-platform test to detect the majority of *DMD* gene mutations and should significantly reduce the time to and cost of diagnosis.[45,47,49]

As discussed, incomplete or nonexhaustive genetic testing can result in patients not receiving a complete or definitive diagnosis. A survey of 41 healthcare professionals (primarily pediatric neurologists and clinical geneticists) showed that although 100% understood the importance of genetic testing, more than 10% would not request further tests if deletions or duplications were not identified.[22] Furthermore, results from the 2017 and 2018 TREAT-NMD Expert Masterclasses on DMD revealed a lack of understanding of DMD genetics (ie, the sequential steps needed to obtain a complete genetic diagnosis for the patient as shown in **Figure 3**) and difficulties with the interpretation of genetic test results (**Table I**, available at www.jpeds.com).

Ultimately, this lack of understanding could result in patients receiving an incomplete or even incorrect genetic diagnosis and prevent them from receiving a mutation-specific therapy for which they are eligible.

Commentary

When asked how long it should take from a specialist ordering the genetic test to the patient receiving a complete genetic diagnosis, the majority of consensus group members indicated that this should take no longer than 8 weeks (77% [10/13]).

Section 2: Recommendations for next steps following a suspected DMD diagnosis (Table III).

Statement 10. Patients with signs and symptoms of DMD and elevated serum CK levels should be referred for genetic testing to either a clinical geneticist or a neuromuscular specialist.

Vote: A+ = 100%; grade of recommendation: 1C

Discussion

No additional supporting information is included.

Commentary

No additional supporting information is included.

Statement 11. A medical/clinical geneticist, a pediatric neurologist, or a neuromuscular specialist should request the genetic test and should provide clinical information relevant to the diagnosis as part of the sample submission to the clinical genetics laboratory, and the genetic diagnostic test should be performed by an accredited laboratory.

Vote: A+ = 100%; grade of recommendation: 1C

Discussion

Laboratories should be formally accredited or certified by organizations such as the International Laboratory Accreditation Cooperation,[29,60] or be covered by such programs as the Clinical Laboratory Improvement Amendments program.[61]

Commentary

The consensus group members were asked who performs the genetic testing in their respective countries. Of the 16 members, 14 indicated that this is performed by a molecular genetics laboratory; however, only 4 of these 14 specified that the laboratory had to be accredited. It was therefore felt that this should be specified in the statement, because it is recommended by current guidelines.[29,61]

Statement 12. Educational meetings for physicians and laboratory specialists on topics relating to the genetic diagnosis of DMD would help to improve the understanding of genetic test reports and the interpretation of genetic test results.

Vote: A+ = 93%; A = 7%; grade of recommendation: 1C

Discussion

In support of this statement, a study of views on genetics training for nongenetic specialists in the United Kingdom showed that of 90 general practitioners surveyed, 90% (81/90) felt that genetics was increasingly important and should be given more attention in their training; 83% (75/90) felt that they did not know all they needed to know about genetics; and 71% (64/90) felt that the training they had received had been insufficient to prepare them for their current role.[62] Similarly, a study of 220 internists from 2 academic medical centers in the US showed that 73.7% and 87.1% of internists rated their knowledge of (1) genetics and (2) genetics guidelines as poor, respectively. The internists also acknowledged that they required further training on particular topics (eg, when to order tests, 79.0%; how to counsel patients, 82.0%; how to interpret results, 77.3%).[63]

Commentary

No additional supporting information is included.

Statement 13. Genetic testing is necessary to inform carrier testing, family planning, genetic counseling, prognosis and optimal management strategies, natural history data gathering, and prenatal diagnosis.

Vote: A+ = 100%; grade of recommendation: 1C

Discussion

Genetic testing is important to inform carrier testing, family planning, and genetic counseling for patients and their families. A qualitative study examined the impact of genetic testing on extended family members. Thirteen grandmothers in families with individuals showing fragile X syndrome or DMD were interviewed. The interviews showed that most grandmothers expressed feelings of guilt or responsibility. This highlighted that although genetic counseling is generally focused on family planning, it also should be offered to extended family members because of the psychosocial impact.[64] Similarly, a qualitative study on parent communication with siblings of children affected by inherited conditions also highlighted the importance of genetic counseling for unaffected siblings, who are at risk of being carriers.[65]

Genetic information is also important for natural history data gathering, because such studies can help to provide information on prognosis and risk of complications; for example, it has been shown that patients with deletions flanking exon 44 or a deletion of exons 3-7 lose ambulation later than those with other out-of-frame deletions (hazard ratio [95% CI]: deletion amenable to exon 44 skipping, 0.34 [0.15-0.74]; $P = .007$; exons 3-7 deletion: 0.24 [0.07-0.82]; $P = .02$).[66-68]

Commentary

No additional supporting information is included.

Statement 14. After a patient receives a complete genetic diagnosis of DMD, it is mandatory that carrier testing of the mother and other at-risk female family members be offered with appropriate pre- and postgenetic counseling (information regarding germline mosaicism and de novo mutations should also be offered). Similarly, testing of other at-risk male family members also should be offered.

Vote: A+ = 100%; grade of recommendation: 1B

Discussion

As per the 2010 best practice guidelines, if carrier status has been confirmed in an individual, genetic counseling should be

offered to that individual and to other at-risk family members. Prenatal screening also may be offered, depending on local practices/legislation.[29] If prenatal testing is offered and returns a positive result, the genetic test report should clearly indicate that the male fetus is predicted to have DMD or BMD. If the mutation identified in the affected patient is not carried by the mother in her somatic cell line, the mother may be a germline mosaic and should be provided with counseling about the risk of having a second son with the disease and that daughters are at risk of being a carrier.[22,29,69] If the mother is found not to be a carrier, the presence of a de novo mutation should be explained; de novo mutations are thought to occur in approximately one-third of isolated cases, and these have been reported throughout the literature.[70-73]

Symptoms of female carriers can include calf pseudohypertrophy; cardiomyopathy; cramps; frequent falls; Gowers' sign; muscle weakness; myalgia[74-77]; and in some cases, intellectual and mental health problems.[75,78,79] A recent study of 36 female DMD and BMD carriers showed that 47% (17/36) had at least 1 pathologic finding on cardiac magnetic resonance imaging, including left ventricular ejection fraction <55% (n = 5 [DMD, n = 4; BMD, n = 1]) and presence of late gadolinium enhancement (n = 16 [DMD, n = 13; BMD, n = 3]).[80] Similarly, a retrospective US observational study of 22 female DMD carriers who underwent cardiac magnetic resonance analysis also showed the presence of cardiac involvement: 18% (4/22) had left ventricular ejection fraction <55%, and 35% (7/20) were found to have late gadolinium enhancement.[81] These findings are supported by a series of case studies and a prospective study of 15 confirmed female carriers.[82-87]

Despite these issues, it is thought that approximately one-third of potential carriers could be unaware of their carrier status[88,89] and may need to be reassessed for risk of carrier status.[90] This is supported by a number of studies. First, a study in the United Kingdom examined levels of carrier testing from 1971 to 2008. In Western Scotland, 843 potential carriers (from 195 families) were tested: of these, 16% and 48% of first-degree and second-degree/more distant relatives had not been tested, respectively. In England, 1223 potential carriers (from 349 families) were tested: of these, 49% of first-degree and 65% of second-degree/more distant relatives had not been tested to determine their carrier status.[91] One study in the Netherlands also showed that of patients registered up to July 1, 2009, 33.7% (35/104) of adult sisters/maternal aunts of patients with DMD who had a 50% risk of being a carrier had not been tested by DNA analysis. This percentage was similar (30.4% [45/148]) for adult sisters with a lower-risk carrier status (4.3% risk).[88] In the US, a recent study by Bogue et al also estimated that 37% of carriers who had an increased risk of cardiomyopathy had never had an echocardiogram and cited that the most commonly identified barrier to carrier testing in the US was the cost of the genetic tests.[89]

As previously discussed, genetic counseling also should be offered for siblings of affected patients, and extended family members (such as grandmothers), to manage the psychosocial impact.[64,65]

Commentary

During the consensus meeting, 93% (14/15) of group members indicated that, at their institute, routine carrier testing was offered to those affected; however, there are geographical differences regarding coverage. Members from the US (n = 4) all indicated that they had experienced problems or had heard of problems when requesting carrier testing, either because insurance companies would not cover the cost of the test or because the physician requesting the test had to provide sufficient rationale to obtain funding to perform it. In contrast, the majority of members from Western Europe and the region of Central and Eastern Europe, Middle East, and Africa indicated that they did not experience difficulties when requesting carrier tests to be performed.

Owing to the difficulties associated with lack of insurance or insufficient insurance coverage, charities and pharmaceutical companies offer or have offered free or subsidized genetic testing for patients and at-risk female relatives; one such initiative is the Decode Duchenne program, set up by Parent Project Muscular Dystrophy. Decode Duchenne offers free genetic testing for individuals with a diagnosis or symptoms of DMD and reduced-rate carrier testing for at-risk female relatives.[92] Based on the findings in the literature and the discussions at the consensus meeting, it was felt that it should be mandatory to offer carrier testing of mothers and other at-risk female relatives (first- and second-degree female relatives, which include the mother of the individual with DMD, along with the individual's female siblings, female offspring, maternal grandmothers, maternal aunts, and their offspring), to provide appropriate medical management.

Statement 15. When a family history is present, presymptomatic CK testing and prenatal testing can lead to earlier detection and thus management of DMD, which is important for family planning.*

Vote: A+ = 87.5%; A = 12.5%; grade of recommendation: 1C

*Where applicable, depending on country-specific legislation on presymptomatic testing patients aged ≤18 years.

Discussion

Although indirectly related, NBS and presymptomatic CK testing have some common discussion points. A report from the 195th European Neuromuscular Centre International Workshop (2012) presented discussions on NBS for DMD; the meeting was attended by 21 experts from 7 countries. The report summarized that although (at the time of the study) there had been 17 pilot NBS programs, no country currently had a national screening program for DMD.[93] The workshop group advised that because of the risk of false-positive and false-negative results with the blood-spot CK test, a 2-tier system should be employed. This approach would use an initial CK test followed by *DMD* gene testing for those with elevated CK levels.[93-95] The feasibility of this approach was demonstrated by Mendell et al.[95,96] Benefits of an NBS program included earlier diagnosis and recognition of previously overlooked symptoms.

During the Canadian NBS program, boys who were diagnosed with DMD were monitored afterwards. In a number of these boys, neurocognitive motor developmental delay was identified at a much earlier age (12-18 months) than would be anticipated.[93] The report also highlighted that physiotherapy and corticosteroid treatment could be implemented earlier as a result of NBS programs.[93] Although only pilot studies have been performed thus far, a NBS program for DMD is being initiated in the Zhejiang Province in China.[97]

Stress and anxiety often are cited as the reasons for not performing NBS for DMD; however, a recent survey of parents of children with DMD, BMD, or spinal muscular atrophy showed that the level of support for NBS for these conditions was >90%.[98] Furthermore, a recent Internet-administered study of 2991 adults showed that participants were more likely to select an optional DMD NBS program when information about this option was presented alongside a mandatory DMD NBS program,[99] highlighting the importance of how these schemes are presented to parents and carers.

A publication by Kwon et al presented a proposed schedule of follow-up visits (and their objectives) for patients identified as having DMD by NBS.[96] The aim of this publication was to provide anticipatory guidance for healthcare professionals, the patients, and the families/guardians to provide effective care for the patient's lifespan after a diagnosis as a result of NBS.

After a genetic diagnosis has been received, prenatal testing also can be performed (depending on local practices) for any at-risk male fetuses[29,100]; this can help to inform any family-planning decisions. Preimplantation screening also is offered at a number of specialist centers.[29]

A study from The Netherlands examined the impact of 26 years of prenatal testing for DMD.[101] The study showed that during the period 1984-2009, 635 prenatal diagnoses were made; of these, 51% were male, and 46% of these male fetuses either were affected by DMD or had an increased risk of the disease. As a result of this prenatal testing, 145 male fetuses were aborted, and 174 continued to full term unaffected by DMD. However, in the cohort of boys during 1961-1974 (n = 397), 62% of the boys did not have an affected relative, suggesting that they were the first affected individual in their family. During the period 1993-2002, 88% of boys in the cohort were the first affected with DMD in their family. The fact that current policy recommends not to test female fetuses, and that many female members are not tested for their carrier status (in this study, 78% of girls ≥16 years old who were at risk of being a carrier had not been tested), has likely contributed to the increased incidence of first affected boys being born.

Commentary

When asked whether CK testing should be offered to younger asymptomatic or presymptomatic male siblings at risk of DMD, 100% of consensus group members who responded answered "yes" (13/13). However, it should be noted that genetic testing in asymptomatic minors is not authorized in some countries. For example, the European Society of Human Genetics indicates that testing of asymptomatic minors is less compelling if the therapeutic measure is deferred to a later time.[102]

The consensus group also was asked if NBS for DMD should be performed: 47% (7/15) members answered "yes"; 47% (7/15) answered "not sure"; and one answered "no." The reason for 47% answering "not sure" was primarily related to the absence of available treatments for patients younger than 5 years of age. However, the earlier treatment of patients with corticosteroids has been shown to be beneficial.[103,104] Lastly, the consensus group members were asked if they felt that CK-based NBS would lead to an earlier genetic diagnosis and improve standards of care, natural history studies, genetic counseling, and family planning: 75% of members (12/16) answered "yes."

The consensus group members were asked whether they felt that prenatal testing for DMD should be offered in the absence of a family history of disease. Of the 16 members, 56% (9/16) answered "no," 31% (5/16) answered "not sure," and 13% (2/16) answered "yes."

Further Reading

1. Guyatt GH, Cook DJ, Sackett DL, Eckman M, Pauker S. Grades of recommendation for antithrombotic agents. Chest 1998;114:441S-444S.
2. Guyatt GH, Oxman AD, Kunz R, Falck-Ytter Y, Vist GE, Liberati A, et al. GRADE: going from evidence to recommendations. BMJ 2008;336:1049-51.
3. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 2008;336:924-6.
4. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Schunemann HJ. GRADE: what is "quality of evidence" and why is it important to clinicians? BMJ 2008;336:995-8.
5. Ciafaloni E, Fox DJ, Pandya S, Westfield CP, Puzhankara S, Romitti PA, et al. Delayed diagnosis in Duchenne muscular dystrophy: data from the Muscular Dystrophy Surveillance, Tracking, and Research Network (MD STARnet). J Pediatr 2009;155:380-5.
6. Wong SH, McClaren BJ, Archibald AD, Weeks A, Langmaid T, Ryan MM, et al. A mixed methods study of age at diagnosis and diagnostic odyssey for Duchenne muscular dystrophy. Eur J Hum Genet 2015;23:1294-300.
7. van Ruiten HJ, Straub V, Bushby K, Guglieri M. Improving recognition of Duchenne muscular dystrophy: a retrospective case note review. Arch Dis Child 2014;99:1074-7.
8. Na SJ, Kim WJ, Kim SM, Lee KO, Yoon B, Choi YC. Clinical, immunohistochemical, Western blot, and genetic analysis in dystrophinopathy. J Clin Neurosci 2013;20:1099-105.
9. Dey S, Senapati AK, Pandit A, Biswas A, Guin DS, Joardar A, et al. Genetic and clinical profile of patients of Duchenne muscular dystrophy: experience from a tertiary care center in Eastern India. Indian Pediatr 2015;52:481-4.
10. Holtzer C, Meaney FJ, Andrews J, Ciafaloni E, Fox DJ, James KA, et al. Disparities in the diagnostic process of Duchenne and Becker muscular dystrophy. Genet Med 2011;13:942-7.
11. Ricotti V, Mandy WP, Scoto M, Pane M, Deconinck N, Messina S, et al. Neurodevelopmental, emotional, and behavioural problems in Duchenne muscular dystrophy in relation to underlying dystrophin gene mutations. Dev Med Child Neurol 2016;58:77-84.
12. Sarrazin E, von der Hagen M, Schara U, von Au K, Kaindl AM. Growth and psychomotor development of patients with Duchenne muscular dystrophy. Eur J Paediatr Neurol 2014;18:38-44.
13. Latimer R, Street N, Conway KC, James K, Cunniff C, Oleszek J, et al. Secondary conditions among males with Duchenne or Becker muscular dystrophy. J Child Neurol 2017;32:663-70.
14. Mirski KT, Crawford TO. Motor and cognitive delay in Duchenne muscular dystrophy: implication for early diagnosis. J Pediatr 2014;165:1008-10.

15. Vicari S, Piccini G, Mercuri E, Battini R, Chieffo D, Bulgheroni S, et al. Implicit learning deficit in children with Duchenne muscular dystrophy: evidence for a cerebellar cognitive impairment? PLoS ONE 2018;13:e0191164.
16. Colombo P, Nobile M, Tesei A, Civati F, Gandossini S, Mani E, et al. Assessing mental health in boys with Duchenne muscular dystrophy: emotional, behavioural and neurodevelopmental profile in an Italian clinical sample. Eur J Paediatr Neurol 2017;21:639-47.
17. Magri F, Govoni A, D'Angelo MG, Del Bo R, Ghezzi S, Sandra G, et al. Genotype and phenotype characterization in a large dystrophinopathic cohort with extended follow-up. J Neurol 2011;258:1610-23.
18. Snow WM, Anderson JE, Jakobson LS. Neuropsychological and neurobehavioral functioning in Duchenne muscular dystrophy: a review. Neurosci Biobehav Rev 2013;37:743-52.
19. Hughes BP. Serum enzyme changes in muscle disease and their relation to tissue change. Proc R Soc Med 1963;56:179-82.
20. Strehle EM, Straub V. Recent advances in the management of Duchenne muscular dystrophy. Arch Dis Child 2015;100:1173-7.
21. Vry J, Gramsch K, Rodger S, Thompson R, Steffensen BF, Rahbek J, et al. European cross-sectional survey of current care practices for Duchenne muscular dystrophy reveals regional and age-dependent differences. J Neuromuscul Dis 2016;3:517-27.
22. Aartsma-Rus A, Ginjaar IB, Bushby K. The importance of genetic diagnosis for Duchenne muscular dystrophy. J Med Genet 2016;53:145-51.
23. European Medicines Agency. Translarna™ summary of product characteristics. https://www.ema.europa.eu/documents/product-information/translarna-epar-product-information_en.pdf. Accessed November 26, 2018.
24. US Food and Drug Administration. EXONDYS 51™ (eteplirsen) injection label. Highlights of prescribing information. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/206488lbl.pdf. Accessed November 26, 2018.
25. US Food and Drug Administration (FDA). FDA News Release: FDA grants accelerated approval to first drug for Duchenne muscular dystrophy. https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm521263.htm. Accessed November 26, 2018.
26. Leiden University Medical Center. Leiden Open Variation Database (LOVD). www.dmd.nl. Accessed November 26, 2018.
27. UMD-DMD and TREAT-NMD. The UMD TREAT-NMD DMD mutations database. http://umd.be/TREAT_DMD/. Accessed November 26, 2018.
28. Institute of Medical Genetics in Cardiff. The Human Gene Mutation Database (HGMD®). http://www.hgmd.cf.ac.uk/ac/index.php. Accessed November 26, 2018.
29. Abbs S, Tuffery-Giraud S, Bakker E, Ferlini A, Sejersen T, Mueller CR. Best practice guidelines on molecular diagnostics in Duchenne/Becker muscular dystrophies. Neuromuscul Disord 2010;20:422-7.
30. Lalic T, Vossen RH, Coffa J, Schouten JP, Guc-Scekic M, Radivojevic D, et al. Deletion and duplication screening in the DMD gene using MLPA. Eur J Hum Genet 2005;13:1231-4.
31. Manjunath M, Kiran P, Preethish-Kumar V, Nalini A, Singh RJ, Gayathri N. A comparative study of mPCR, MLPA, and muscle biopsy results in a cohort of children with Duchenne muscular dystrophy: a first study. Neurol India 2015;63:58-62.
32. Khordadpoor-Deilamani F, Akbari MT, Nafissi S, Zamani G. Dystrophin gene mutation analysis in Iranian males and females using multiplex polymerase chain reaction and multiplex ligation-dependent probe amplification methods. Genet Test Mol Biomarkers 2011;15:893-9.
33. Dastur RS, Kachwala MY, Khadilkar SV, Hegde MR, Gaitonde PS. Identification of deletions and duplications in the Duchenne muscular dystrophy gene and female carrier status in western India using combined methods of multiplex polymerase chain reaction and multiplex ligation-dependent probe amplification. Neurol India 2011;59:803-9.
34. Xu Y, Wang H, Xiao B, Wei W, Liu Y, Ye H, et al. Novel noncontiguous duplications identified with a comprehensive mutation analysis in the *DMD* gene by *DMD* gene-targeted sequencing. Gene 2018;645:113-8.
35. Kim MJ, Cho SI, Chae JH, Lim BC, Lee JS, Lee SJ, et al. Pitfalls of multiple ligation-dependent probe amplifications in detecting DMD exon deletions or duplications. J Mol Diagn 2016;18:253-9.
36. Santos R, Goncalves A, Oliveira J, Vieira E, Vieira JP, Evangelista T, et al. New variants, challenges and pitfalls in DMD genotyping: implications in diagnosis, prognosis and therapy. J Hum Genet 2014;59:454-64.
37. Araujo APQC, Carvalho AAS, Cavalcanti EBU, Saute JAM, Carvalho E, Franca MCJ, et al. Brazilian consensus on Duchenne muscular dystrophy. Part 1: diagnosis, steroid therapy and perspectives. Arq Neuropsiquiatr 2017;75:104-13.
38. Bovolenta M, Neri M, Fini S, Fabris M, Trabanelli C, Venturoli A, et al. A novel custom high density-comparative genomic hybridization array detects common rearrangements as well as deep intronic mutations in dystrophinopathies. BMC Genomics 2008;9:572.
39. Baskin B, Gibson WT, Ray PN. Duchenne muscular dystrophy caused by a complex rearrangement between intron 43 of the *DMD* gene and chromosome 4. Neuromuscul Disord 2011;21:178-82.
40. Ishmukhametova A, Khau Van Kien P, Mechin D, Thorel D, Vincent MC, Rivier F, et al. Comprehensive oligonucleotide array-comparative genomic hybridization analysis: new insights into the molecular pathology of the *DMD* gene. Eur J Hum Genet 2012;20:1096-100.
41. Hegde MR, Chin EL, Mulle JG, Okou DT, Warren ST, Zwick ME. Microarray-based mutation detection in the dystrophin gene. Hum Mutat 2008;29:1091-9.
42. Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. Lancet Neurol 2010;9:77-93.
43. Birnkrant DJ, Bushby K, Bann CM, Apkon SD, Blackwell A, Brumbaugh D, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. Lancet Neurol 2018;17:251-67.
44. Wang Y, Yang Y, Liu J, Chen XC, Liu X, Wang CZ, et al. Whole dystrophin gene analysis by next-generation sequencing: a comprehensive genetic diagnosis of Duchenne and Becker muscular dystrophy. Mol Genet Genomics 2014;289:1013-21.
45. Alame M, Lacourt D, Zenagui R, Mechin D, Danton F, Koenig M, et al. Implementation of a reliable next-generation sequencing strategy for molecular diagnosis of dystrophinopathies. J Mol Diagn 2016;18:731-40.
46. Bladen CL, Salgado D, Monges S, Foncuberta ME, Kekou K, Kosma K, et al. The TREAT-NMD DMD Global Database: analysis of more than 7,000 Duchenne muscular dystrophy mutations. Hum Mutat 2015;36:395-402.
47. Vasli N, Bohm J, Le Gras S, Muller J, Pizot C, Jost B, et al. Next generation sequencing for molecular diagnosis of neuromuscular diseases. Acta Neuropathol 2012;124:273-83.
48. Lim BC, Lee S, Shin JY, Kim JI, Hwang H, Kim KJ, et al. Genetic diagnosis of Duchenne and Becker muscular dystrophy using next-generation sequencing technology: comprehensive mutational search in a single platform. J Med Genet 2011;48:731-6.
49. Flanigan KM, von Niederhausern A, Dunn DM, Alder J, Mendell JR, Weiss RB. Rapid direct sequence analysis of the dystrophin gene. Am J Hum Genet 2003;72:931-9.
50. Wei X, Dai Y, Yu P, Qu N, Lan Z, Hong X, et al. Targeted next-generation sequencing as a comprehensive test for patients with and female carriers of DMD/BMD: a multi-population diagnostic study. Eur J Hum Genet 2014;22:110-8.
51. Roucher Boulez F, Menassa R, Streichenberger N, Manel V, Mallet-Motak D, Morel Y, et al. A splicing mutation in the DMD gene detected by next-generation sequencing and confirmed by mRNA and protein analysis. Clin Chim Acta 2015;448:146-9.
52. Magri F, Del Bo R, D'Angelo MG, Govoni A, Ghezzi S, Gandossini S, et al. Clinical and molecular characterization of a cohort of patients with novel nucleotide alterations of the dystrophin gene detected by direct sequencing. BMC Med Genet 2011;12:37.

53. Niba ETE, Nishida A, Tran VK, Vu DC, Matsumoto M, Awano H, et al. Cryptic splice activation but not exon skipping is observed in minigene assays of dystrophin c.9361 + 1G>A mutation identified by NGS. J Hum Genet 2017;62:531-7.
54. Wang Z, Lin Y, Qiu L, Zheng D, Yan A, Zeng J, et al. Hybrid minigene splicing assay verified the pathogenicity of a novel splice site variant in the dystrophin gene of a Chinese patient with typical Duchenne muscular dystrophy phenotype. Clin Chem Lab Med 2016;54:1435-40.
55. Baskin B, Stavropoulos DJ, Rebeiro PA, Orr J, Li M, Steele L, et al. Complex genomic rearrangements in the dystrophin gene due to replication-based mechanisms. Mol Genet Genomic Med 2014;2:539-47.
56. Khelifi MM, Ishmukhametova A, Khau Van Kien P, Thorel D, Mechin D, Perelman S, et al. Pure intronic rearrangements leading to aberrant pseudoexon inclusion in dystrophinopathy: a new class of mutations? Hum Mutat 2011;32:467-75.
57. Zaum AK, Stuve B, Gehrig A, Kolbel H, Schara U, Kress W, et al. Deep intronic variants introduce DMD pseudoexon in patient with muscular dystrophy. Neuromuscul Disord 2017;27:631-4.
58. Tuffery-Giraud S, Miro J, Koenig M, Claustres M. Normal and altered pre-mRNA processing in the *DMD* gene. Hum Genet 2017;136:1155-72.
59. Greer K, Mizzi K, Rice E, Kuster L, Barrero RA, Bellgard MI, et al. Pseudoexon activation increases phenotype severity in a Becker muscular dystrophy patient. Mol Genet Genomic Med 2015;3:320-6.
60. The International Laboratory Accreditation Cooperation (ILAC). http://ilac.org/. Accessed November 26, 2018.
61. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24.
62. Burke S, Stone A, Bedward J, Thomas H, Farndon P. A "neglected part of the curriculum" or "of limited use"? Views on genetics training by nongenetics medical trainees and implications for delivery. Genet Med 2006;8:109-15.
63. Klitzman R, Chung W, Marder K, Shanmugham A, Chin LJ, Stark M, et al. Attitudes and practices among internists concerning genetic testing. J Genet Couns 2013;22:90-100.
64. Lehmann A, Speight BS, Kerzin-Storrar L. Extended family impact of genetic testing: the experiences of X-linked carrier grandmothers. J Genet Couns 2011;20:365-73.
65. Plumridge G, Metcalfe A, Coad J, Gill P. Parents' communication with siblings of children affected by an inherited genetic condition. J Genet Couns 2011;20:374-83.
66. Bello L, Morgenroth LP, Gordish-Dressman H, Hoffman EP, McDonald CM, Cirak S, et al. DMD genotypes and loss of ambulation in the CINRG Duchenne natural history study. Neurology 2016;87:401-9.
67. Bello L, Kesari A, Gordish-Dressman H, Cnaan A, Morgenroth LP, Punetha J, et al. Genetic modifiers of ambulation in the Cooperative International Neuromuscular Research Group Duchenne natural history study. Ann Neurol 2015;77:684-96.
68. van den Bergen JC, Ginjaar HB, Niks EH, Aartsma-Rus A, Verschuuren JJ. Prolonged ambulation in Duchenne patients with a mutation amenable to exon 44 skipping. J Neuromuscul Dis 2014;1:91-4.
69. Bermudez-Lopez C, Garcia-de Teresa B, Gonzalez-del Angel A, Alcantara-Ortigoza MA. Germinal mosaicism in a sample of families with Duchenne/Becker muscular dystrophy with partial deletions in the *DMD* gene. Genet Test Mol Biomarkers 2014;18:93-7.
70. Garcia S, de Haro T, Zafra-Ceres M, Poyatos A, Gomez-Capilla JA, Gomez-Llorente C. Identification of *de novo* mutations of Duchenne/Becker muscular dystrophies in southern Spain. Int J Med Sci 2014;11:988-93.
71. Strmecki L, Hudler P, Benedik-Dolnicar M, Komel R. De novo mutation in DMD gene in a patient with combined hemophilia A and Duchenne muscular dystrophy. Int J Hematol 2014;99:184-7.
72. Luna-Angulo AB, Gomez-Diaz B, Escobar-Cedillo RE, Anaya-Segura MA, Estrada-Mena FJ, Lopez-Hernandez LB. A new de novo mutation in a non-hot spot region at the DMD gene in a Mexican family. Genet Couns 2014;25:429-32.
73. Li T, Zhang ZJ, Ma X, Lv X, Xiao H, Guo QN, et al. Prenatal diagnosis for a Chinese family with a de novo *DMD* gene mutation: a case report. Medicine (Baltimore) 2017;96:e8814.
74. Juan-Mateu J, Rodriguez MJ, Nascimento A, Jimenez-Mallebrera C, Gonzalez-Quereda L, Rivas E, et al. Prognostic value of X-chromosome inactivation in symptomatic female carriers of dystrophinopathy. Orphanet J Rare Dis 2012;7:82.
75. Papa R, Madia F, Bartolomeo D, Trucco F, Pedemonte M, Traverso M, et al. Genetic and early clinical manifestations of females heterozygous for Duchenne/Becker muscular dystrophy. Pediatr Neurol 2016;55:58-63.
76. Nozoe KT, Akamine RT, Mazzotti DR, Polesel DN, Grossklauss LF, Tufik S, et al. Phenotypic contrasts of Duchenne muscular dystrophy in women: two case reports. Sleep Sci 2016;9:129-33.
77. Takeshita E, Minami N, Minami K, Suzuki M, Awashima T, Ishiyama A, et al. Duchenne muscular dystrophy in a female with compound heterozygous contiguous exon deletions. Neuromuscul Disord 2017;27:569-73.
78. Imbornoni L, Price ET, Andrews J, Meaney FJ, Ciafaloni E, Cunniff C. Diagnostic and clinical characteristics of early-manifesting females with Duchenne or Becker muscular dystrophy. Am J Med Genet A 2014;164A:2769-74.
79. Mercier S, Toutain A, Toussaint A, Raynaud M, de Barace C, Marcorelles P, et al. Genetic and clinical specificity of 26 symptomatic carriers for dystrophinopathies at pediatric age. Eur J Hum Genet 2013;21:855-63.
80. Florian A, Rosch S, Bietenbeck M, Engelen M, Stypmann J, Waltenberger J, et al. Cardiac involvement in female Duchenne and Becker muscular dystrophy carriers in comparison to their first-degree male relatives: a comparative cardiovascular magnetic resonance study. Eur Heart J Cardiovasc Imaging 2016;17:326-33.
81. Lang SM, Shugh S, Mazur W, Sticka JJ, Rattan MS, Jefferies JL, et al. Myocardial fibrosis and left ventricular dysfunction in Duchenne muscular dystrophy carriers using cardiac magnetic resonance imaging. Pediatr Cardiol 2015;36:1495-501.
82. Parent JJ, Moore RA, Taylor MD, Towbin JA, Jefferies JL. Left ventricular noncompaction cardiomyopathy in Duchenne muscular dystrophy carriers. J Cardiol Cases 2015;11:7-9.
83. Schelhorn J, Schoenecker A, Neudorf U, Schemuth H, Nensa F, Nassenstein K, et al. Cardiac pathologies in female carriers of Duchenne muscular dystrophy assessed by cardiovascular magnetic resonance imaging. Eur Radiol 2015;25:3066-72.
84. Cheng VE, Prior DL. Peripartum cardiomyopathy in a previously asymptomatic carrier of Duchenne muscular dystrophy. Heart Lung Circ 2013;22:677-81.
85. Martinez HR, Pignatelli R, Belmont JW, Craigen WJ, Jefferies JL. Childhood onset of left ventricular dysfunction in a female manifesting carrier of muscular dystrophy. Am J Med Genet A 2011;155A:3025-9.
86. Yilmaz A, Gdynia HJ, Ludolph AC, Klingel K, Kandolf R, Sechtem U. Images in cardiovascular medicine. Cardiomyopathy in a Duchenne muscular dystrophy carrier and her diseased son: similar pattern revealed by cardiovascular MRI. Circulation 2010;121:e237-9.
87. Walcher T, Kunze M, Steinbach P, Sperfeld AD, Burgstahler C, Hombach V, et al. Cardiac involvement in a female carrier of Duchenne muscular dystrophy. Int J Cardiol 2010;138:302-5.
88. Helderman-van den Enden ATJM, van den Bergen JC, Breuning MH, Verschuuren JJGM, Tibben A, Bakker E, et al. Duchenne/Becker muscular dystrophy in the family: have potential carriers been tested at a molecular level? Clin Genet 2011;79:236-42.
89. Bogue L, Peay H, Martin A, Lucas A, Ramchandren S. Knowledge of carrier status and barriers to testing among mothers of sons with Duchenne or Becker muscular dystrophy. Neuromuscul Disord 2016;26:860-4.
90. Bogue L, Ramchandren S. Outdated risk assessment in a family with Duchenne dystrophy: implications for duty to reassess. Neurol Genet 2016;2:e103.

91. McGowan R, Challoner BR, Ross S, Holloway S, Joss S, Wilcox D, et al. Results of Duchenne muscular dystrophy family screening in practice: leaks rather than cascades? Clin Genet 2013;83:187-90.
92. DuchenneConnect. DuchenneConnect: DecodeDuchenne—frequently asked questions. https://www.duchenneconnect.org/component/content/article/114-website-content/decodeprogram/961-faq.html. Accessed November 26, 2018.
93. Ellis JA, Vroom E, Muntoni F. 195th ENMC International Workshop: newborn screening for Duchenne muscular dystrophy December 14-16, 2012, Naarden, The Netherlands. Neuromuscul Disord 2013;23:682-9.
94. Moat SJ, Bradley DM, Salmon R, Clarke A, Hartley L. Newborn bloodspot screening for Duchenne muscular dystrophy: 21 years experience in Wales (UK). Eur J Hum Genet 2013;21:1049-53.
95. Mendell JR, Shilling C, Leslie ND, Flanigan KM, al-Dahhak R, Gastier-Foster J, et al. Evidence-based path to newborn screening for Duchenne muscular dystrophy. Ann Neurol 2012;71:304-13.
96. Kwon JM, Abdel-Hamid HZ, Al-Zaidy SA, Mendell JR, Kennedy A, Kinnett K, et al. Clinical follow-up for Duchenne muscular dystrophy newborn screening: a proposal. Muscle Nerve 2016;54:186-91.
97. Ke Q, Zhao ZY, Griggs R, Wiley V, Connolly A, Kwon J, et al. Newborn screening for Duchenne muscular dystrophy in China: follow-up diagnosis and subsequent treatment. World J Pediatr 2017;13:197-201.
98. Wood MF, Hughes SC, Hache LP, Naylor EW, Abdel-Hamid HZ, Barmada MM, et al. Parental attitudes toward newborn screening for Duchenne/Becker muscular dystrophy and spinal muscular atrophy. Muscle Nerve 2014;49:822-8.
99. Lillie SE, Tarini BA, Janz NK, Zikmund-Fisher BJ. Framing optional genetic testing in the context of mandatory newborn screening tests. BMC Med Inform Decis Mak 2015;15:50.
100. Wang H, Xu Y, Liu X, Wang L, Jiang W, Xiao B, et al. Prenatal diagnosis of Duchenne muscular dystrophy in 131 Chinese families with dystrophinopathy. Prenat Diagn 2017;37:356-64.
101. Helderman-van den Enden AT, Madan K, Breuning MH, van der Hout AH, Bakker E, de Die-Smulders CE, et al. An urgent need for a change in policy revealed by a study on prenatal testing for Duchenne muscular dystrophy. Eur J Hum Genet 2013;21:21-6.
102. ESHG. Genetic testing in asymptomatic minors: recommendations of the European Society of Human Genetics. Eur J Hum Genet 2008;17:720-1.
103. Merlini L, Cicognani A, Malaspina E, Gennari M, Gnudi S, Talim B, et al. Early prednisone treatment in Duchenne muscular dystrophy. Muscle Nerve 2003;27:222-7.
104. Merlini L, Gennari M, Malaspina E, Cecconi I, Armaroli A, Gnudi S, et al. Early corticosteroid treatment in 4 Duchenne muscular dystrophy patients: 14-year follow-up. Muscle Nerve 2012;45:796-802.

Table I. Example survey results from the 2017 and 2018 TREAT-NMD*,† Expert Masterclasses on DMD regarding the genetic diagnosis of patients with DMD

Question	Correct answer	TREAT-NMD Expert Masterclass	Total number of responses, N	Number who answered correctly, n (%)	Number who answered incorrectly, n (%)	Number who answered "I do not know," n (%)
MLPA analysis is negative for a patient with suspected DMD. What is your next step? (1) Multiplex PCR, (2) Consider another differential diagnosis, (3) Sequencing of all exons and flanking sequences, (4) Muscle biopsy and dystrophin analysis, (5) I do not know.	Sequencing of all exons and flanking sequences	2017	64	51 (79.7)	12 (18.8)‡	1 (1.6)
		2018	45	40 (88.9)	5 (11.1)§	0 (0.0)
MLPA analysis reveals a deletion of exon 20. What is your next step? (1) Multiplex PCR, (2) Perform a second test to confirm the finding, (3) Nothing, the diagnosis is confirmed, (4) Muscle biopsy and dystrophin analysis, (5) I do not know.	Perform a second test to confirm the finding	2017	64	31 (48.4)	31 (**48.4**)	2 (3.1)
		2018	45	12 (26.7)	31 (**68.9**)	2 (4.4)
A patient with a deletion of exon 51 is eligible for which therapy? (1) Ataluren/stop-codon readthrough, (2) Eteplirsen/exon-51 skipping, (3) No therapy yet available, (4) I do not know.	No therapy yet available	2017	65	18 (27.7)	39 (**60.0**)	8 (12.3)
		2018	49	21 (42.9)	20 (**40.8**)	8 (16.3)
Multiplex analysis reveals the presence of exons 44, 45, and 51, and the absence of exon 50. Is this patient eligible for exon 51 skipping? (1) Yes, (2) No, (3) I do not know.	I do not know	2017	57	14 (24.6)	43 (**75.4**)	NA
		2018	46	12 (26.1)	34 (**73.9**)	NA

CGH, comparative genome hybridization; *DMD*, Duchenne muscular dystrophy; *MLPA*, multiplex ligation-dependent probe amplification; *NA*, not applicable; *PCR*, polymerase chain reaction.
Questions answered incorrectly by at least 25% of respondents have been highlighted.
*2017 TREAT-NMD Expert Masterclass, May 18-19, 2017, Lisbon, Portugal (TREAT-NMD received an unrestricted educational grant from PTC Therapeutics International, Ltd, for the organization of this meeting). 2018 TREAT-NMD Expert Masterclass organized and funded by PTC Therapeutics International, Ltd, in collaboration with TREAT-NMD, May 2-3, 2018, Madrid, Spain.
†2017 TREAT-NMD Expert Masterclass: 61 attendees provided information on their job roles (child neurologist, n = 24; other, n = 11; trainee doctor, n = 7; neurologist, n = 5; physiotherapist, n = 5; neurology nurse, n = 4; pediatrician, n = 2; geneticist, n = 2, and pediatric nurse, n = 1). 2018 TREAT-NMD Expert Masterclass: 39 attendees provided information on their job roles (child neurologist, n = 15; other, n = 14; pediatrician, n = 5; neurologist, n = 4; and pediatric nurse, n = 1).
‡Seven (10.9%) selected muscle biopsy.
§Four (8.9%) selected muscle biopsy.

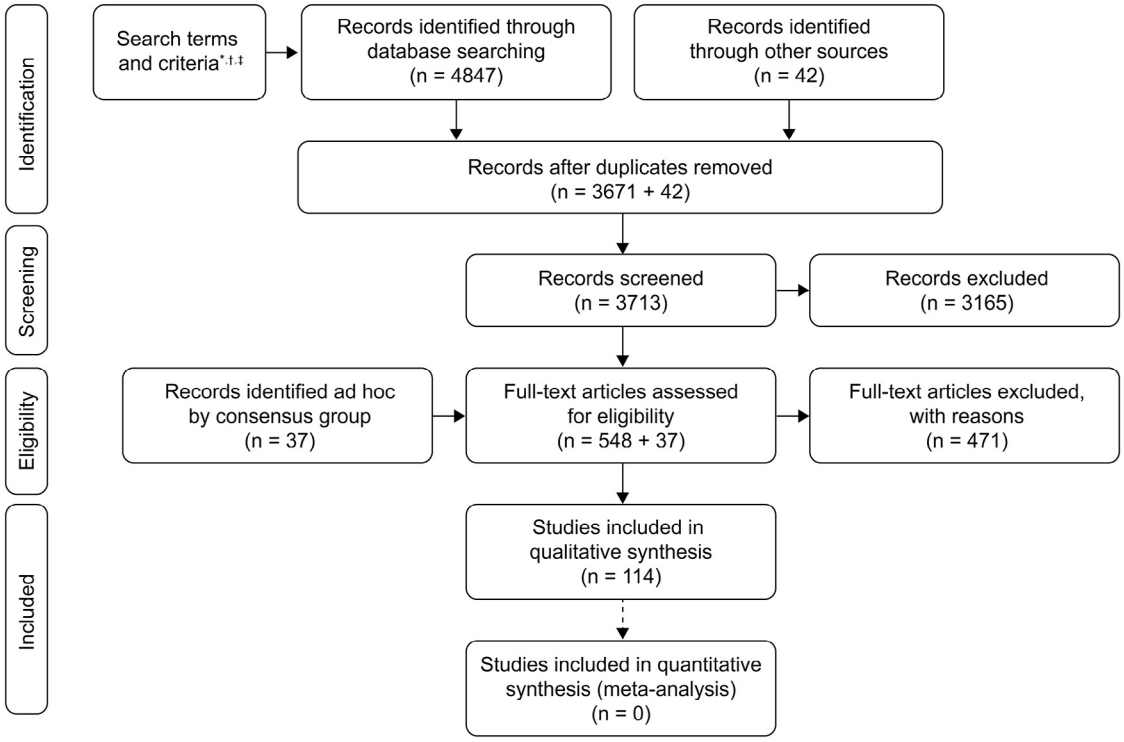

Figure 2. PRISMA flow diagram of systematic literature searches. The searches were conducted using Ovid to screen the EMBASE and MEDLINE databases. *PCR*, polymerase chain reaction. *Disease search terms (limited to title/abstract): Duchenne muscular dystrophy, DMD, or Duchenne AND any of the following search terms (limited to title/abstract): sign; symptom; creatine kinase; transaminase; diagnos*; gene*; genetic test*; genetic report*; genetic counsel*; delay*; screen*; carrier; mutation; sequenc*; comparative genome hybridi?ation; CGH; multiplex ligation-dependent probe amplification; MLPA; multiplex polymerase chain reaction; multiplex PCR; biops*; prenatal; neonatal; female; family planning; germline mosaicism; de novo; practitioner; p?ediatrician; p?ediatric neurologist; neuromuscular specialist. The "?" function was included to search for variations of the spelling; the "*" function was included to search for variations of the word; and search terms were limited to the title or abstract of articles only. The full electronic search strategy is presented in **Table II**. †Search criteria: articles published from January 1, 2010, to April 8, 2018; English language; studies of humans; full journal articles. ‡Relevant articles older than 2010 (if no newer reference could be found) were identified ad hoc by the steering committee and expert voting panel.

Table II. Electronic search strategy for MEDLINE and EMBASE databases, performed in Ovid (2017 Ovid Technologies, Inc)

Lines	Search
1	Duchenne muscular dystrophy.tw
2	DMD.tw
3	Duchenne.tw
4	1 or 2 or 3
5	sign.tw
6	symptom.tw
7	creatine kinase.tw
8	transaminase.tw
9	diagnos*.tw
10	gene*.tw
11	genetic test.tw
12	genetic report.tw
13	genetic counsel*.tw
14	delay*.tw
15	screen*.tw
16	carrier.tw
17	mutation.tw
18	sequenc*.tw
19	comparative genome hybridi?ation.tw
20	CGH.tw
21	multiplex ligation-dependent probe amplification.tw
22	MLPA.tw
23	multiplex polymerase chain reaction.tw
24	multiplex PCR.tw
25	biops*.tw
26	prenatal.tw
27	neonatal.tw
28	female.tw
29	family planning.tw
30	germline mosaicism.tw
31	de novo.tw
32	practitioner.tw
33	p?ediatrician.tw
34	p?ediatric neurologist.tw
35	neuromuscular specialist.tw
36	5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35
37	4 and 36
38*	limit 37 to dd=20100101-20170621 [Limit not valid in Ovid MEDLINE(R),Ovid MEDLINE(R) Daily Update,Ovid MEDLINE(R) In-Process,Ovid MEDLINE(R) Publisher; records were retained]
39*	limit 38 to ed="20100101-20170621" [Limit not valid in Embase; records were retained]
40	limit 39 to English language
41	limit 40 to human
42	Limit 41 to humans
43	Remove duplicates from 42

*The first search was performed from January 1, 2010, to June 21, 2017; an update was then performed from June 22, 2017, to April 8, 2018, using the same strategy.
Search results were then exported, and full journal articles selected, using Excel's filter function.