Genetics in Medicine (2024) 26, 101145

ELSEVIER

Genetics in Medicine
An Official Journal of the ACMG

www.journals.elsevier.com/genetics-in-medicine

ACMG TECHNICAL STANDARD

Myotonic dystrophy type 1 testing, 2024 revision: A technical standard of the American College of Medical Genetics and Genomics (ACMG)

Bryce A. Seifert[1], Honey V. Reddi[2], Benjamin E. Kang[3,4], Lora J.H. Bean[5], Amy Shealy[6], Nancy C. Rose[7]; on behalf of the ACMG Laboratory Quality Assurance Committee[8,*]

Disclaimer: This technical standard is designed primarily as an educational resource for clinical laboratory geneticists to help them provide quality clinical laboratory genetic services. Adherence to this technical standard is voluntary and does not necessarily assure a successful medical outcome. This technical standard should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, clinical laboratory geneticists should apply their own professional judgment to the specific circumstances presented by the individual patient or specimen.

Clinical laboratory geneticists are encouraged to document the rationale for how a particular test was designed, its intended use and its performance specifications as well as whether or not it is in conformance with this technical standard. They also are advised to take notice of the date any particular technical standard was adopted, and to consider other relevant medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures. Where individual authors are listed, the views expressed may not reflect those of authors′ employers or affiliated institutions.

ARTICLE INFO

Article history:
Received 11 April 2024
Accepted 11 April 2024
Available online 5 June 2024

Keywords:
Myotonic dystrophy
Technical standard
Testing methodologies
Trinucleotide repeats

ABSTRACT

Myotonic dystrophy type 1 (DM1) is a form of muscular dystrophy causing progressive muscle loss and weakness. Although clinical features can manifest at any age, it is the most common form of muscular dystrophy with onset in adulthood. DM1 is an autosomal dominant condition, resulting from an unstable CTG expansion in the 3′-untranslated region of the myotonic dystrophy protein kinase (*DMPK*) gene. The age of onset and the severity of the phenotype are roughly correlated with the size of the CTG expansion. Multiple methodologies can be used to diagnose affected individuals with DM1, including polymerase chain reaction, Southern blot, and triplet repeat-primed polymerase chain reaction. Recently, triplet repeat interruptions have been described, which may affect clinical outcomes of a fully-variable allele in *DMPK*. This document supersedes the Technical Standards and Guidelines for Myotonic Dystrophy originally published in 2009 and reaffirmed in 2015. It is designed for genetic testing professionals who are already familiar with the disease and the methods of analysis.

Current affiliation for Honey Reddi: Belay Diagnostics, Chicago, IL

The Board of Directors of the American College of Medical Genetics and Genomics approved this technical standard on 15 March 2024.

*Correspondence: ACMG. *Email address:* documents@acmg.net
Affiliations are at the end of the document.

doi: https://doi.org/10.1016/j.gim.2024.101145

1. Introduction

This document updates the standards for myotonic dystrophy type 1 (DM1) originally published in 2009[1] and reaffirmed in 2015.[2] The current revision updates the methodology section to include triplet repeat-primed polymerase chain reaction (PCR) and discusses the use of next-generation sequencing (NGS) and its technological limitations for detection of myotonic dystrophy protein kinase (*DMPK*) CTG expansions, adds a section on Human Genome Variation Society (HGVS) nomenclature in accordance with current technical standards, uses the term "DM1 alleles" instead of "mutation" with respect to either variable or fully-variable alleles ("variable allele" for the premutation variant and "fully-variable allele" for the full mutation variant), and discusses the clinical utility of CCG and other interruptions within the *DMPK* CTG expansion. This document is designed for genetic testing professionals and is not intended as a clinical practice guideline.

2. Background on Myotonic Dystrophy Type 1

2.1. Gene symbol/chromosome locus and OMIM number

Myotonic dystrophy protein kinase (*DMPK*) gene (HUGO Gene Nomenclature Committee (HGNC) ID: HGNC:2933) at chromosome 19q13.32 (OMIM number 605377).

2.2. Brief clinical description

Myotonic dystrophy type 1 (DM1, MIM 160900) is a multisystem disorder characterized by progressive muscle weakness, myotonia, intellectual impairment, cataracts, cardiac arrhythmias, respiratory insufficiency, hypogonadism, and endocrine disturbances.[3] DM1 generally causes distal muscle weakness that is progressive. Because of the wide range and severity of symptoms, the diagnosis may be elusive. The severe congenital form of DM1 results in developmental delay, respiratory distress, hypotonia, with an increased risk for neonatal death because of respiratory complications. Those who survive the neonatal period initially follow a static course, eventually learning to walk but with significant development delay and intellectual disability. The congenital form is most often observed in the offspring of affected women, although the disorder may not be identified in the proband until after the birth of a congenitally affected child. For more information, see the online *GeneReviews* e-book (NBK1165).[3]

Myotonic dystrophy type 2 (DM2, MIM 602668), previously termed proximal myotonic myopathy, is often milder than DM1 and affects the central musculature.[4] DM2 occurs due to a CCTG expansion located in intron 1 of the zinc finger protein 9 gene (*ZNF9*, HGNC approved symbol: *CNBP*; HGNC:13164) on chromosome 3q21.3.[5] Individuals affected with DM2 also have a complex clinical presentation that is similar to DM1, including myotonia, cardiac involvement, cataracts, hypogonadism, insulin insensitivity, and histological abnormalities in skeletal muscle. Individuals with DM2 can often be distinguished from those with DM1 by a milder course with more proximal muscle weakness, sparing of facial muscles, and the absence of learning disabilities. The severe congenital form is not associated with DM2. For more information, see the myotonic dystrophy type 2 *GeneReviews* (NBK1466).[4]

2.3. Mode of inheritance

Inheritance for DM1 is autosomal dominant with nearly complete penetrance but variable expressivity; some individuals remain undiagnosed because of mild symptoms.

2.4. Gene description/normal gene product

The recommended Matched Annotation from NCBI and EMBL-EBI (MANE) Select (http://tark.ensembl.org/web/mane_project/)[6] transcript (NM_004409.5; ENST00000291270.9) for reporting variants in the *DMPK* gene spans approximately 13 kb and contains 15 exons. *DMPK* encodes a serine-threonine kinase, which is expressed in all tissues affected in DM1. Transcription of the gene results in the production of several alternatively spliced forms. There is differential expression of the alternatively spliced isoforms in different tissues.

2.5. DM1 alleles

DMPK is the only known gene associated with DM1. A DM1 allele is defined as any expansion of the CTG trinucleotide repeat located in the 3′ untranslated region (UTR) of the *DMPK* gene, corresponding to the last exon,[7] that is beyond the normal range of CTG repeats.[8-10] The number of CTG repeats varies in the normal population from 5 to 34, and within this range, the alleles are stably transmitted. Individuals with 35 to 49 repeats (variable alleles) do not have symptoms but their children are at an increased risk of inheriting larger repeats and having symptoms. When the repeat length is at least 50 CTGs (in some individuals up to several thousands), the allele becomes unstable and results in the DM1 phenotype (fully-variable alleles). Variants at locations other than the CTG expansion in the 3′ UTR of the gene have not been described in DM1.

2.5.1. Definition of normal and variant categories of the *DMPK* CTG repeats

Three main allelic ranges exist within *DMPK*, with the associated number of CTG repeats determined by current knowledge to date. These ranges are represented in ClinVar[11] under submission IDs: SCV001424573.1, SCV001424572.1, SCV001424571.1.

2.5.1.1. Normal allele

Normal alleles have a range of ~5 to 34 repeats. These alleles are not pathologic and segregate as stable polymorphic repeats. The distribution of normal alleles is not random. A trimodal distribution is observed in most populations, with the most frequently occurring allele being 5 repeats. The second mode consists of 3 major alleles of copy numbers 11, 12, and 13 and a minor allele of 14 repeats. The final mode has no clear peak but represents alleles of 19 repeats or more.[12]

2.5.1.2. Variable allele

Variable alleles have a range of ~35 to 49 repeats. Variable alleles are often identified in distant relatives of probands with fully-variable alleles. Variable alleles are not associated with a clinical phenotype in the heterozygote but are unstable and liable to expand in succeeding generations.

2.5.1.3. Fully-variable allele

Fully-variable alleles are defined by 50 or more repeats. These repeats are unstable and are associated with disease manifestations.

2.5.1.4. Mosaicism

Individuals with DM1 often show marked somatic mosaicism of the CTG repeat. The expansions often give a diffuse or smeared appearance on Southern blotting. Heterogeneity within and differences in average repeat lengths between tissues in the same individual has been confirmed. Somatic mosaicism in various tissues may be a probable explanation for the lack of a correlation between the repeat size of DNA from blood lymphocytes and clinical symptoms in some cases. In one study, CTG expansions were 2-to-13-fold greater in DNA isolated from skeletal muscle than in DNA isolated from leukocytes in 10 of 11 individuals with DM1.[13]

2.6. Genotype/phenotype association

There is a significant correlation between CTG repeat size, age of onset, and clinical severity.[14,15] Mildly affected individuals have 50 to 100 repeats and may only report cataracts and/or mild myotonia. More classically affected individuals have ~100 to ~1000 CTG repeats, and congenital cases often have 1000 to 6000 CTG repeats (Table 1). When comparing unrelated affected individuals with small to moderate differences in repeat sizes, it is difficult to accurately predict the severity of the disease. This is because of overlap between triplet repeat size and severity of the disease, including the degree or presence of organ involvement, in affected individuals.[13] However, a significant increase in allele size in a child compared with the parent confers a high likelihood of earlier onset and a more severe phenotype.[14] To account for the maintenance of expanded alleles in the population, it was proposed that there is a high incidence of minimally expanded alleles in DM1 families, which produce few symptoms and are stably transmitted over several generations.

Assessment of genotype-phenotype correlation is complicated by dynamic somatic mosaicism of the CTG repeat, which is biased toward continuous expansion throughout the lifetime of an affected individual that is age- and size-dependent.[16] The trinucleotide is mitotically and meiotically unstable with a bias toward length increase in the next generation accounting for "anticipation" or increasing severity in successive generations of the same family, with earlier age of onset. Although repeat expansions occur through both maternal and paternal transmissions, the larger repeat expansions observed in congenital cases are almost exclusively due to maternal transmissions. Several cases of reverse alleles (ie, contraction to normal range of repeats) have also been reported in DM1, whereby there is a spontaneous reversion of an expanded allele upon transmission to an unaffected offspring. The mechanism for the DM1 reverse alleles remains unknown. A gene conversion mechanism, whereby the normal parental allele replaces the expanded allele, or a double recombination event leading to a disruption of the CTG repeats have been proposed as possible mechanisms for spontaneous contractions. The reversions may provide an explanation of the nonpenetrance observed in some DM1

Table 1 Association between CTG repeat lengths, category, and clinical significance

# CTG Repeats	Variant Category/Disease Form	Clinical Significance
~5-34	Normal	No disease association. Alleles are transmitted stably with low risk of CTG expansion in the next generation.
35-49	Variable allele	No disease association. Increased risk of CTG repeat expansion in the next generation.
50-100	Fully-variable allele/Mild disease	Consistent with a diagnosis of myotonic dystrophy type 1. Mild symptoms can include cataracts and/or mild myotonia.
~100-~1000	Fully-variable allele/Classic disease	Consistent with a diagnosis of myotonic dystrophy type 1. Classic symptoms can include progressive muscle weakness, myotonia, intellectual impairment, cataracts, cardiac arrhythmias, respiratory insufficiency, hypogonadism, and endocrine disturbances.
1000-6000	Fully-variable allele/Congenital disease	Consistent with a diagnosis of myotonic dystrophy type 1. Congenital symptoms can include developmental delay, respiratory distress, hypotonia, and often death shortly after birth due to respiratory complications.

families. Therefore, challenges for the genetic counseling of individuals and families affected by DM1 include extreme clinical variability, somatic mosaicism, anticipation, influence of sex of the affected individual, and nonpenetrance because of reversions.

2.7. Mutational mechanism

The CTG repeat is located within the 3′ UTR of a gene that encodes the myotonin protein kinase. Because the repeat is not in the protein coding portion (ie, the exons), the molecular mechanism by which the fully-variable allele exerts its dominant expression is difficult to understand and describe. It has been suggested that the myotonin kinase mRNA with long CUG repeats, and not the protein, result in a gain-of-function RNA pathogenesis.[17-19] Novel RNA-binding proteins that specifically bind to CUG repeats may be depleted by excessive CUG repeats in the DM1 transcripts. Muscleblind-like protein-1 and CUG-binding protein-1 are 2 RNA-binding proteins proposed to be involved in the pathogenesis. The depletion of these CUG-binding proteins has been shown to cause splicing alterations of the chloride channel-1 gene and the insulin receptor genes, resulting in myotonia and insulin resistance, phenotypes that are related to the clinical features of DM1.

2.8. Interruptions in DM1 alleles

Besides the well-defined CTG repeats in the *DMPK* gene, variant repeats interrupted by CCG, CGG, CAG, or CTC have been identified at the 3′ end of *DMPK*,[20-26] which are collectively known as "variant repeats with interruptions". Variations in fully-variable alleles are observed wherein interruptions occur in about 3% to 8% of individuals affected with DM1,[27] with the most common interruption being the presence of CCG interruptions.[28] It has been demonstrated that the interruptions act as *cis*-acting modifiers with a stabilizing effect on *DMPK* expansions and are prone to relatively stable intergenerational transmission or even contractions of *DMPK* expansions independent of the sex of the transmitting parent in many studies.[20-23,29]

Many studies have reported that individuals with interruptions manifest disease at a later age of onset and with a milder phenotype than those with a corresponding size of the pure CTG repeat expansions with no interruptions.[30,31] Individuals with interruptions in their fully-variable alleles showed a lower level of somatic instability and epigenetic DNA methylation change in the *DMPK* locus with mild symptoms and slower progression of the disease.[24,32,33] However, individuals with interruptions may exhibit atypical patterns of symptoms.[20-24] Also, no congenital or childhood forms of DM1 have been reported in individuals with fully-variable alleles that contain interruptions.[28]

Considering the type of interruptions (CCG, CGG, CAG, or CTC), pattern, length of interruptions, and location within the expansion, the phenotypic consequence of fully-variable alleles with interruptions is still uncertain, and further studies are needed to define the genotype-phenotype correlation of the presence of interruptions. Therefore, it is not recommended to predict the prognosis of DM1 based on the simple detection of a fully-variable allele with interruptions in the *DMPK* gene.

2.9. Incidence and ethnic association of the DM1 variant

The prevalence of DM1 varies among different geographic regions and ethnicities; the estimated worldwide prevalence of DM1 is 1/5000-1/20,000.[34] In regions of Quebec, the prevalence of DM1 is approximately 1/550 because of a founder effect.

3. Methods

This laboratory technical standard was informed by a review of the literature and expert opinion. We consulted PubMed (search terms included: myotonic dystrophy, triplet repeat-primed PCR and *DMPK*, interruptions in *DMPK*, myotonic dystrophy type 1 variant repeat expansion, *DMPK* variant repeat with interruptions, *DMPK* interruption, and *DMPK* repeat expansion with variant interruption), the American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines for Clinical Genetics Laboratories,[35] Clinical Laboratory Improvement Amendments regulations, Online Mendelian Inheritance in Man (OMIM), and *GeneReviews*. When conflicting information arose within the literature, the authors used expert opinion to inform the document. Expert opinion included the authors of the document and members of the Molecular Genetics Subcommittee of the Laboratory Quality Assurance Committee. Any conflicts of interest for workgroup members are listed at the end of the article. The ACMG Laboratory Quality Assurance Committee reviewed the document providing further input on the content, and a final draft was delivered to the ACMG Board of Directors for review and approval to send out for member comment. The final draft of the document was posted on the ACMG website, and an email link was sent to ACMG members inviting all to provide comments. All member comments were assessed by the authors and our recommendations were amended as deemed appropriate. Member comments and author responses were reviewed by a representative of the Laboratory Quality Assurance Committee and the ACMG Board of Directors. The final document was approved by the ACMG Board of Directors.

4. Testing Considerations

4.1. Analytical sensitivity and analytical specificity

Fully-variable alleles account for >99% of cases of DM1; therefore, the analytical sensitivity and specificity of tests

that effectively detect and measure the CTG repeat in the 3′ UTR of *DMPK* approaches 100%. However, fully-variable alleles in the range of 50 to 100 repeats can be identified in asymptomatic or minimally affected individuals. Therefore, factors such as age, family history, penetrance, and variable expressivity preclude an accurate determination of the clinical sensitivity and specificity of the test in individuals with 1 or both alleles in this range of repeats. For CTG repeats >100, the clinical sensitivity and specificity is high and expected to approach 100%. Allele sizes of 35 to 49 CTG repeats (variable alleles) are rare and have been mostly ascertained through symptomatic relatives, with >50 repeats.

4.2. Indications for genetic testing

Testing for *DMPK* CTG repeat expansions is often used for symptomatic confirmatory diagnostic testing, testing those who exhibit equivocal symptoms, such as isolated cataracts, and predictive testing of asymptomatic individuals after the identification of a fully-variable allele in affected family members. The test is also used for prenatal diagnosis of at-risk pregnancies with sonographic findings, including fetal hypotonia, or positional abnormalities with hydramnios. Prenatal diagnosis in both amniotic fluid cells and chorionic villus samples can be performed; maternal cell contamination studies should be done to confirm the fetal origin of the sample.

5. Guidelines

5.1. Methodological considerations

US laboratories offering molecular diagnostic testing for DM1 must follow all federal and state regulations relevant to clinical laboratory operations. This includes meeting all Clinical Laboratory Improvement Amendments and, if applicable to the laboratory, College of American Pathologists (CAP) quality control requirements. In addition, all laboratories must be active participants in biannual DM1 proficiency testing challenges. All methodological applications should also follow the current ACMG Technical Standards for Clinical Genetics Laboratories[35] developed by the ACMG Laboratory Quality Assurance Committee. Non-US laboratories are expected to be similarly compliant with their individual countries statutory regulations governing oversight of clinical laboratories.

The direct DNA tests have reduced the number of invasive (muscle biopsy) and minimally invasive (electromyography) diagnostic techniques for the diagnosis of DM1. The combination of Southern blot and PCR can detect all DM1 alleles.[36] The majority of clinically significant DM1 alleles can be identified by Southern blot analysis; however, for small amplifications (<100 repeats), PCR is essential. Although the PCR test is less expensive and faster than the Southern blot, longer repeats are often not reliably amplified. General guidelines for Southern blot analysis and PCR are provided in the ACMG Technical Standards for Clinical Genetics Laboratories.[35] The following additional details are specific for DM1 molecular testing (Figure 1, Table 2). Reference materials exist that possess the normal range of CTG repeats (5 to 34 repeats), variable range (35-49 repeats), and fully-variable number of CTG repeats (50 repeats).[37] These materials are publicly available from the Centers for Disease Control and Prevention Genetic Testing Reference Materials Coordination Program (https://www.cdc.gov/labquality/get-rm/index.html) and the Coriell Cell Repositories (https://www.coriell.org/).

5.2. Southern blot analysis

5.2.1. Probes

Several probes are available for hybridization, including PGB2.6, pMDY1, cDNA25, and p5B1.4.[9,10,38,39] Several of the probes will detect not only the DM1 alleles but also an Alu repeat insertion/deletion change within the restriction fragment. It has been shown that the insertion allele is almost in complete linkage disequilibrium with the DM1 alleles, suggesting that the disease appeared to be the result of 1 or a few ancestral variants.

5.2.2. Restriction enzymes

For the best resolution of smaller expansions, a restriction enzyme should be chosen that allows the probe to hybridize to a smaller fragment. By reducing the size of the restriction fragment, resolution is improved, and expansions as small as 100 repeats can easily be detected. Figure 2 shows a Southern blot analysis with DNA, from several individuals, digested with *HindIII* and *BglI* run on 0.8% agarose gel and blotted on a nylon membrane. Increasing the electrophoretic migration will also improve the detection of smaller expansions. The background may interfere with the detection of the larger expanded alleles because the expanded alleles often appear as diffuse smears because of the somatic instability of the variant. The efficiency of the detection of these somatically variable expansions can be increased by the application of a relatively infrequent cutting enzyme that will generate a larger restriction fragment containing the expansion. *EcoRI* cuts a large 9 to 10 kb fragment and *HindIII* cuts an 8.5 to 9.5 kb fragment in the DM1 gene. The larger fragment lengths will reduce the smearing effect of the somatic variability of the unstable repeats. Decreasing the electrophoretic migration will lead to better band definition, resulting in an improved detection of larger somatic mosaic expansions as distinct bands instead of diffuse smears. Therefore, the most efficient approach for identifying expansions may be a combination of different restriction enzymes and variation in electrophoretic duration.[40,41] Expansion sizes can be estimated from Southern blots by using a standard ladder, such as lambda *HindIII* fragments or a set of chosen controls.

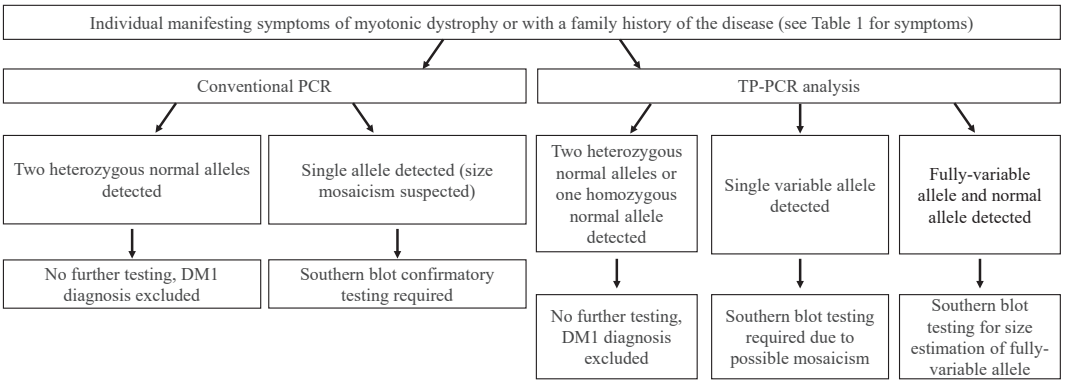

Figure 1 Diagnostic testing algorithm for myotonic dystrophy type 1. Ranges of *DMPK* CTG repeats are as follows: normal alleles (~5 to 34 CTG repeats), variable alleles (~35 to 49 CTG repeats), fully-variable alleles (50 or more CTG repeats). PCR, polymerase chain reaction; TP-PCR, triplet repeat-primed PCR.

5.3. PCR methods

Several sets of primers, PCR conditions, amplicon separation, and detection techniques have been published. Regardless of the particular PCR-based strategy selected, it is important that assay conditions and post-PCR analyses be optimized to ensure for the accurate and unambiguous sizing of repeat lengths.

5.3.1. Conventional PCR

Conventional PCR can be used to detect smaller expansions and are usually observed in milder cases, which are difficult to resolve by Southern blotting. However, longer repeats are not reliably detected by PCR; therefore, the method is not suitable to make a direct diagnosis in many cases. PCR can be useful in excluding DM1, when individuals demonstrate 2 different normal size alleles. When 2 normal alleles are identified, the DM1 diagnosis can be excluded, and Southern blot testing is not necessary. However, because the heterozygosity frequency for the CTG repeats is ~75% in the normal population, ~25% of unaffected individuals will be homozygous for a given normal allele. Therefore, the presence of a single PCR band does not confirm a diagnosis of DM1. All single bands require Southern blot confirmation.

Accurate sizing of repeat lengths should be empirically determined by comparison with appropriate external or internal standards. These could include, but are not limited to, (M13) sequencing ladders, cloned reference standards, and appropriate normal and abnormal controls, whose sizes have been independently verified. For each analysis, appropriate controls that include a range of CTG sizes should be used. It is the responsibility of the laboratory to empirically determine the detection limits for their assays.

5.3.2. Triplet repeat-primed PCR

In triplet repeat-primed PCR (TP-PCR) analysis, primers complementary to the *DMPK* CTG repeats that bind randomly to the repeat are used in combination with a primer outside the repeat, enabling the amplification of repeats of varying sizes and resulting in a pool of DNA fragments. Downstream size separation of these fragments results in a stutter pattern of variable repeat sizes (Figure 3). Size separation typically involves capillary electrophoresis, and the coupling of TP-PCR with capillary electrophoresis has been described in multiple studies for clinical testing of DM1.[25,26,42-46] Assay validation should include determination of performance characteristics outlined by regulatory agencies and the ACMG Technical Standards for Clinical Genetics Laboratories.[35]

A range of repeat sizes should be run to estimate the outer limits of expansion detection because larger repeats may not be detected.[42] Laboratories are encouraged to determine the sensitivity/limit of detection of their assay using DNA with a normal *DMPK* allele titrated with serial dilutions of DNA containing fully-variable *DMPK*. When a homozygous peak is detected well within the expected normal range (~5-34), additional testing is usually not required, unless mosaicism is suspected (eg, an individual has mild symptoms), in which case Southern blot is necessary (Figure 1, Table 2). In other instances that a single peak is detected, particularly for those peaks that lie in the variable allele range (~35-49 repeats), additional analyses, such as Southern blot testing, may be necessary to rule out size mosaicism for a variable allele and fully-variable allele, bearing in mind that size mosaicism can vary between tissue types, and the CTG repeats in *DMPK* exhibit mitotic instability (see Section 2.5 for details concerning mosaicism). Size mosaicism has been reported to be detected down to ~10%.[25]

Controls should be used as part of the clinical workflow and include a no DNA control, a sensitivity control, and a rotating pool of positive case samples or verified reference materials[37] containing repeats in the normal, variable, and fully-variable ranges. Additionally, a sensitivity control should be included in each run wherein a normal sample is diluted with a sample containing a fully-variable allele. Refer to Section 5.1 for information on reference materials.

Variant repeats with interruptions affect melting and amplification of template DNA because of increases in GC

Table 2 Suggested comments for reporting myotonic dystrophy type 1 results

Variant	Interpretation	Clinical Significance for the Affected Individual	Clinical Significance for the Affected Individual's Family	Recommendations	Testing Recommendations to Clarify Conventional PCR or TP-PCR Results
Normal allele	The individual's *DMPK* alleles contain (***) and (***) CTG repeats, consistent with the normal range (5-34 repeats).	Myotonic dystrophy type 1 is not associated with *DMPK* alleles within the normal range.	Myotonic dystrophy type 1 is not associated with *DMPK* alleles within the normal range.	Genetic counseling is recommended	Southern blot is recommended if mosaicism is suspected (eg, mild clinical symptoms) and in instances of single bands detected by conventional PCR.
Variable allele	The individual's *DMPK* alleles contain (***) and (***) CTG repeats, consistent with the variable allele range (35-49 repeats).	Variable alleles are not associated with myotonic dystrophy phenotypes but are liable to expand in future generations.	Variable alleles are not associated with myotonic dystrophy phenotypes but are liable to expand in future generations. Although repeat expansions occur through both paternal and maternal transmissions, larger repeat expansions observed in congenital cases are almost exclusively due to maternal transmissions.	Genetic counseling and *DMPK* DNA testing are recommended for at-risk relatives to determine the size of their *DMPK* allele(s). Prenatal diagnosis in future pregnancies should be considered.	Southern blot is recommended because of possible mosaicism for variable alleles and fully-variable alleles.
Fully-variable allele	This individual possesses a fully-variable allele of greater than or equal to 50 CTG repeats in *DMPK*. Southern blot detected a repeat size of (***) CTG repeats.	This result is consistent with a diagnosis of myotonic dystrophy type 1.	Parents of children with fully-variable *DMPK* alleles are either heterozygous for a variable allele and at risk of having affected children or are heterozygous for a fully-variable allele which can cause symptoms in the parent. Individuals with fully-variable alleles are at 50% risk of transmitting the fully-variable allele to their children.	Genetic counseling and *DMPK* DNA testing are recommended for at-risk relatives to determine the size of their *DMPK* allele(s). Prenatal diagnosis in future pregnancies should be considered.	Southern blot may be needed for detection of larger repeat sizes.

PCR, polymerase chain reaction; *TP-PCR*, triplet repeat-primed PCR.

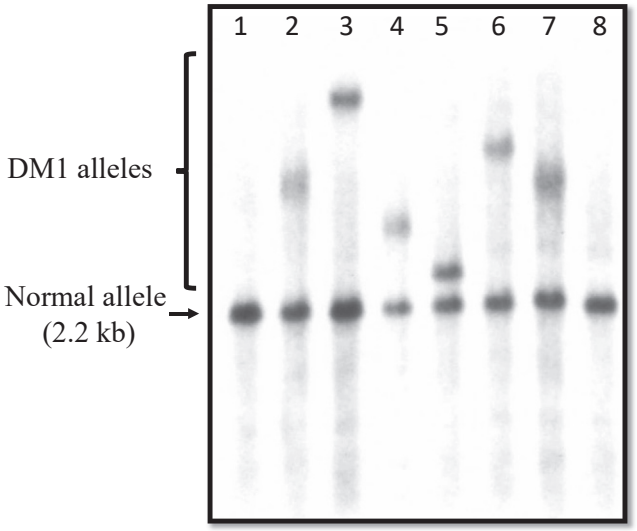

Figure 2 Southern blot detection of the CTG expansion in *DMPK*. *HindIII-BglI*-digested genomic DNA probed with pMDY1. Samples in lanes 1 and 8 are unaffected controls. Samples from patients with DM1 (lanes 2-7) show an expanded fragment representing a DM1 allele. The normal allele is 2.2 kb.

content, producing inaccurate results because of allele dropout of the abnormal allele.[24-26] The use of primers with different fluorescent labels can reduce false-negatives from allele dropout of abnormal alleles.[26] It is currently not recommended to report variant repeats with interruptions in *DMPK*, given that the clinical utility is not yet established. See Section 2.8 for additional details regarding this recommendation.

6. Alternative Methodologies

6.1. Next-generation sequencing

Although recent advances in NGS technology and bioinformatics tools[26,31] have enabled the detection of repeat expansions within *DMPK* and its DNA methylation pattern, this technology is not routinely used within clinical laboratories for detection and reporting of these repeats. Short-read NGS in its current state cannot reliably detect expanded *DMPK* alleles and is not recommended at this time. Long-read sequencing can detect expanded *DMPK* alleles and mosaic alleles; however, it is not routinely used in clinical laboratories for detection of expanded repeats.[24,47]

6.2. Small-pool PCR

Small-pool PCR involves the amplification of a nucleotide repeat element, such as a trinucleotide repeat, in many small pools of input DNA containing a range of genome inputs.[48] Although this methodology is able to detect somatic mosaicism for *DMPK* CTG repeats, it is mostly used in research settings and not routinely offered as a clinical test.[49-54] Therefore, small-pool PCR is not recommended at this time as a clinical test for detection of repeat expansions in *DMPK*.

6.3. Nomenclature for reporting of DM1 alleles

The use of standard nomenclature is important for the accurate communication of results to health care providers and is recommended by the ACMG and CAP in accordance with HGVS recommendations.[55] According to HGVS, regarding the nomenclature for short sequence repeats, the nomenclature for CTG expansions within the 3′ UTR of *DMPK* should be listed in the general format of: NM_004409.5:c.[*224_*283CTG["copy_number"]];[*224_*283CTG["copy_number"]] with "copy_number" indicating the number of repeat units and a semicolon used to describe variants on different chromosomes. For the genomic DNA (g.) description, the nomenclature should be listed in the general format of: NC_000019.10:g.[45770205_45770264GAC["copy_number"]];[45770205_45770264GAC["copy_number"]]. When 2 variants are detected, but it is not known whether these variants exist on the same or different chromosomes, "(;)" should be used.[53] Examples include:

NM_004409.5:c.[*224_*283CTG[5]];[*224_*283CTG[11]] for an individual that is compound heterozygous for a normal allele with 5 CTG repeats and a normal allele of 11 CTG repeats.

NM_004409.5:c.*224_*283CTG[35](;)*224_*283CTG[5] for an individual that has a variable allele of 35 CTG repeats and a normal allele of 5 CTG repeats. Here, it is not known whether these variants are on one chromosome (in *cis*) or on different chromosomes (in *trans*).

NM_004409.5:c.[*224_*283CTG[50]];[*224_*283CTG[5]] for an individual that is compound heterozygous for a fully-variable allele of 50 CTG repeats and a normal allele of 5 CTG repeats.

7. Interpretations

Elements considered essential to the reporting of clinical test results are described in detail in the current ACMG Standards and Guidelines for Clinical Genetics Laboratories.[35] The following additional elements must also be included in the reporting of the DM1 genotype (see Table 2 for additional information).

- The methodology used to assign the genotype: If PCR methodology is used, then a description of the primer pair(s) should be included, as well as the method of amplicon separation and detection. If a Southern blot was required, the restriction enzyme(s) and probe(s) should be identified. Each report must state the CTG

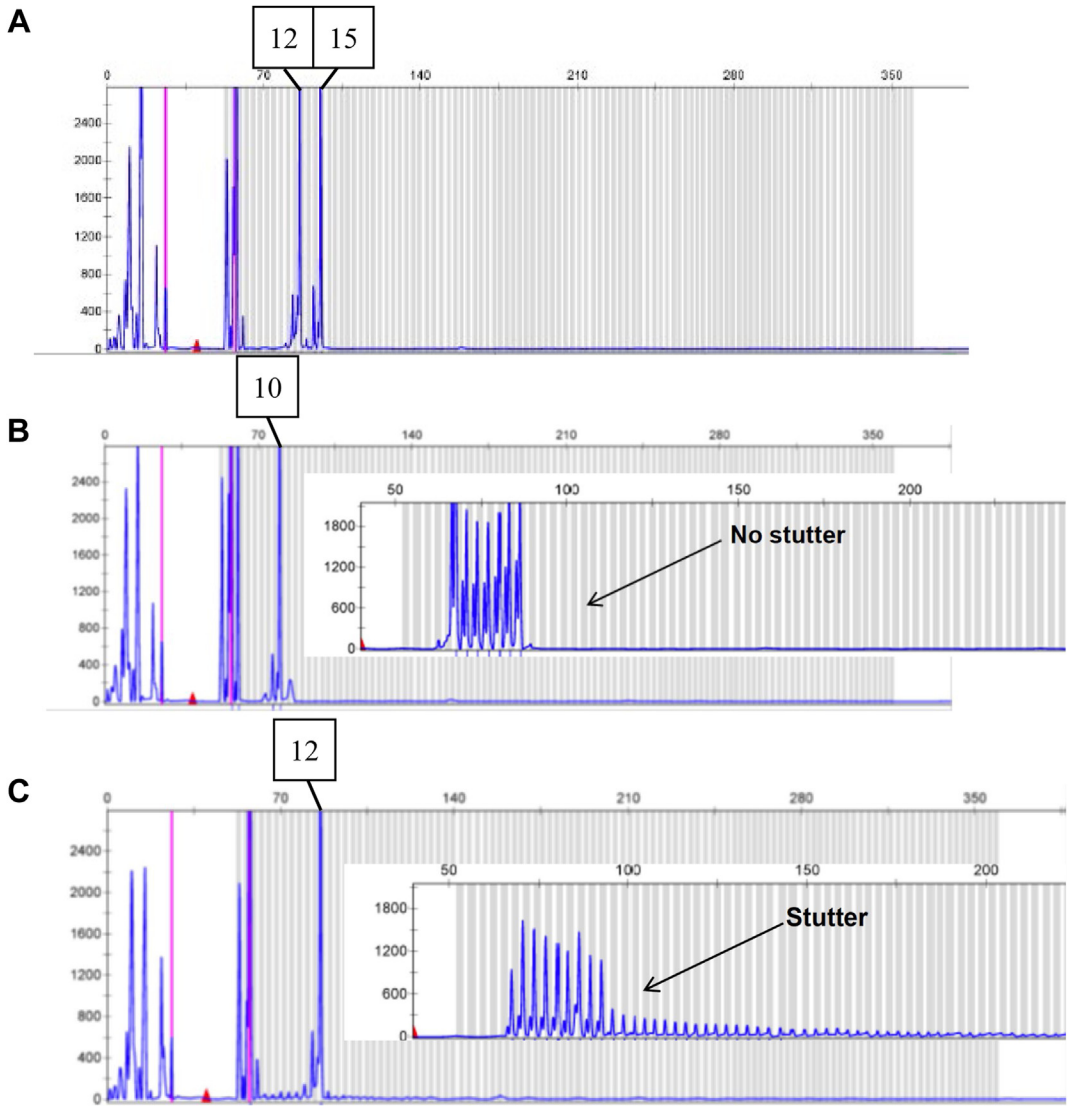

Figure 3 Triplet repeat-primed PCR results. A. Individual with 12 and 15 CTG repeats in *DMPK*. CTG repeat sizes are indicated above each peak. B. Individual that is homozygous for 10 CTG repeats. The inset depicts the absence of a stutter pattern. C. Individual with 12 CTG repeats and a fully-variable allele in *DMPK* (50 or more CTG repeats). The inset depicts a stutter pattern, consistent with a fully-variable allele (50 or more CTG repeats) in *DMPK*.

repeat length categories and descriptors currently used in clinical practice, and each reportable genotype should be classified and interpreted using these categorical definitions.

- The normal, variable, and fully-variable repeat ranges should be clearly stated in the report. Each report must include the CTG repeat numbers of both alleles with the precision of sizing required for accurate clinical interpretation (see Section 2.5). Laboratories may confirm their precision based on consensus results of proficiency testing programs, such as the one offered by CAP. For large alleles determined by Southern blot, qualifying terms, such as "approximately" or "estimated" can be used but the alleles should be clearly described in the interpretive comments to prevent any unnecessary ambiguity. All positive results should state that genetic counseling is indicated, and testing is available for other at-risk family members.
- Informed consent should be addressed, including issues with respect to accurate paternity, possible diagnostic errors due to sampling or labeling errors, and genotype inaccuracies due to the presence of rare polymorphisms.
- The following statement must be included in the report. "This test was developed and its performance characteristics determined by this laboratory. It has not been cleared or approved by the US Food and Drug Administration (21 CFR §809.30[e]). The Food and Drug Administration has determined that such clearance or approval is not necessary. This analysis is used for clinical purposes. It should not be regarded as investigational or for research."

- The following alternative diagnosis may be included: Proximal DM2 will not be detected by this test.
- Comments on phenotype, if included, should be abstract rather than case specific. The following concepts apply.
  - For asymptomatic testing, the a priori risk of inheriting a fully-variable allele is modified by DM1 CTG repeat analysis. However, predictions from the repeat size regarding degree of severity or age of onset should not be included in the report.
  - For prenatal diagnosis, because of the overlapping ranges and uncertainty regarding somatic mosaicism and in utero instability of the expanded CTG repeat, it is not possible to predict whether the fetus will have the congenital or the adult-onset form of DM1.

8. Conclusion

Myotonic dystrophy type 1 is caused by expansion of CTG repeats in the 3′ UTR of *DMPK*. Reduced penetrance and variable expressivity exist within the disorder, largely due to meiotic and mitotic instability of CTG repeats, and genetic anticipation. Additionally, variant repeats with interruptions can affect the ability to accurately detect abnormal alleles and the phenotypic consequence of fully-variable alleles with interruptions remains uncertain. These technical standards serve as a guide for clinical DM1 testing that should be applied to any methodology used. With advances in TP-PCR, NGS, and other technologies, the approach to testing for DM1 is likely to change as technologies become more advanced in the detection of repeat expansions in *DMPK*.

Acknowledgments

The authors wish to thank Yassmine Akkari, PhD, for her contribution of results from TP-PCR on *DMPK*.

Peter Freeman, Lecturer in Healthcare Sciences (Clinical bioinformatics, genomics) at the University of Manchester, *GIM* and *GIMO* Senior Tecnhnical Editor. PI of VariantValidator and HUGO Reporting of Sequence Variants committee member. The *GIM* technical editing team and VariantValidator team are dedicated to delivering robust representation of genomics data in biomedical journals and are proud to be working with the ACMG on their publications to set the highest possible standards for the benefit of patients.

Author Information

Project administration: B.A.S., H.V.R.; Writing-original draft: B.A.S., H.V.R., B.E.K., L.J.H.B, A.S., N.C.R.; Writing-review & editing: B.A.S., H.V.R., B.E.K., L.J.H.B., A.S., N.C.R.

Conflict of Interest

Honey V. Reddi, Benjamin E. Kang, and Lora J.H. Bean all serve as directors in clinical laboratories that perform a breadth of genetic and genomic analyses on a fee-for-service basis. Honey V. Reddi is a Consultant Director for Biofidelity Inc. Bryce A. Seifert is a salaried employee of Guidehouse, LLP. All other authors declare no conflicts of interest.

Affiliations

[1]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; [2]Department of Pathology and Laboratory Medicine, Medical College of Wisconsin, Milwaukee, WI; [3]Department of Pathology and Pediatrics, University of Michigan Medical School, Ann Arbor, MI; [4]Vanderbilt University Medical Center, Nashville, TN; [5]Revvity Omics, Pittsburgh, PA; [6]Cleveland Clinic Center for Personalized Genetic Healthcare, Cleveland, OH; [7]Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, University of Utah, Salt Lake City, UT; [8]American College of Medical Genetics and Genomics, Bethesda, MD

References

1. Prior TW. Technical standards and guidelines for myotonic dystrophy type 1 testing. *Genet Med*. 2009;11(7):552-555. http://doi.org/10.1097/GIM.0b013e3181abce0f
2. Prior TW. Addendum: technical standards and guidelines for myotonic dystrophy type 1 testing. *Genet Med*. 2016;18(1):107. http://doi.org/10.1038/gim.2015.176
3. Bird TD. Myotonic dystrophy type 1. In: Adam MP, Mirzaa GM, Pagon RA, et al., eds. *GeneReviews*. Seattle: University of Washington; September 17, 1999. Last revision March 21, 2024.
4. Schoser B. Myotonic dystrophy type 2. In: Adam MP, Mirzaa GM, Pagon RA, et al., eds. *GeneReviews*. Seattle: University of Washington; September 21, 2006. Last update March 19, 2020.
5. Liquori CL, Ricker K, Moseley ML, et al. Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science*. 2001;293(5531):864-867. http://doi.org/10.1126/science.1062125
6. Morales J, Pujar S, Loveland JE, et al. A joint NCBI and EMBL-EBI transcript set for clinical genomics and research. *Nature*. 2022;604(7905):310-315. http://doi.org/10.1038/s41586-022-04558-8
7. Turner C, Hilton-Jones D. Myotonic dystrophy: diagnosis, management and new therapies. *Curr Opin Neurol*. 2014;27(5):599-606. http://doi.org/10.1097/WCO.0000000000000128
8. Brook JD, McCurrach ME, Harley HG, et al. Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member. *Cell*. 1992;68(4):799-808. http://doi.org/10.1016/0092-8674(92)90154-5
9. Fu YH, Pizzuti A, Fenwick RG Jr, et al. An unstable triplet repeat in a gene related to myotonic muscular dystrophy. *Science*. 1992;255(5049):1256-1258. http://doi.org/10.1126/science.1546326

10. Mahadevan M, Tsilfidis C, Sabourin L, et al. Myotonic dystrophy mutation: an unstable CTG repeat in the 3′ untranslated region of the gene. *Science*. 1992;255(5049):1253-1255. http://doi.org/10.1126/science.1546325
11. Landrum MJ, Lee JM, Riley GR, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014;42(database issue):D980-D985. http://doi.org/10.1093/nar/gkt1113
12. Martorell L, Monckton DG, Sanchez A, Lopez De Munain A, Baiget M. Frequency and stability of the myotonic dystrophy type 1 premutation. *Neurology*. 2001;56(3):328-335. http://doi.org/10.1212/wnl.56.3.328
13. Thornton CA, Johnson K, Moxley RT 3rd. Myotonic dystrophy patients have larger CTG expansions in skeletal muscle than in leukocytes. *Ann Neurol*. 1994;35(1):104-107. http://doi.org/10.1002/ana.410350116
14. Redman JB, Fenwick RG Jr, Fu YH, Pizzuti A, Caskey CT. Relationship between parental trinucleotide GCT repeat length and severity of myotonic dystrophy in offspring. *JAMA*. 1993;269(15):1960-1965. http://doi.org/10.1001/jama.1993.03500150072029
15. Tsilfidis C, MacKenzie AE, Mettler G, Barceló J, Korneluk RG. Correlation between CTG trinucleotide repeat length and frequency of severe congenital myotonic dystrophy. *Nat Genet*. 1992;1(3):192-195. http://doi.org/10.1038/ng0692-192
16. Wong LJ, Ashizawa T, Monckton DG, Caskey CT, Richards CS. Somatic heterogeneity of the CTG repeat in myotonic dystrophy is age and size dependent. *Am J Hum Genet*. 1995;56(1):114-122.
17. Ranum LPW, Day JW. Myotonic dystrophy: RNA pathogenesis comes into focus. *Am J Hum Genet*. 2004;74(5):793-804. http://doi.org/10.1086/383590
18. Charlet-Berguerand N, Savkur RS, Singh G, Philips AV, Grice EA, Cooper TA. Loss of the muscle-specific chloride channel in type 1 myotonic dystrophy due to misregulated alternative splicing. *Mol Cell*. 2002;10(1):45-53. http://doi.org/10.1016/S1097-2765(02)00572-5
19. Savkur RS, Philips AV, Cooper TA. Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy. *Nat Genet*. 2001;29(1):40-47. http://doi.org/10.1038/ng704
20. Musova Z, Mazanec R, Krepelova A, et al. Highly unstable sequence interruptions of the CTG repeat in the myotonic dystrophy gene. *Am J Med Genet A*. 2009;149A(7):1365-1374. http://doi.org/10.1002/ajmg.a.32987
21. Braida C, Stefanatos RKA, Adam B, et al. Variant CCG and GGC repeats within the CTG expansion dramatically modify mutational dynamics and likely contribute toward unusual symptoms in some myotonic dystrophy type 1 patients. *Hum Mol Genet*. 2010;19(8):1399-1412. http://doi.org/10.1093/hmg/ddq015
22. Botta A, Rossi G, Marcaurelio M, et al. Identification and characterization of 5′ CCG interruptions in complex DMPK expanded alleles. *Eur J Hum Genet*. 2017;25(2):257-261. http://doi.org/10.1038/ejhg.2016.148
23. Pešović J, Perić S, Brkušanin M, Brajušković G, Rakočević-Stojanović V, Savić-Pavićević D. Molecular genetic and clinical characterization of myotonic dystrophy type 1 patients carrying variant repeats within DMPK expansions. *Neurogenetics*. 2017;18(4):207-218. http://doi.org/10.1007/s10048-017-0523-7
24. Santoro M, Masciullo M, Pietrobono R, et al. Molecular, clinical, and muscle studies in myotonic dystrophy type 1 (DM1) associated with novel variant CCG expansions. *J Neurol*. 2013;260(5):1245-1257. http://doi.org/10.1007/s00415-012-6779-9
25. Leferink M, Wong DPW, Cai S, et al. Robust and accurate detection and sizing of repeats within the DMPK gene using a novel TP-PCR test. *Sci Rep*. 2019;9(1):8280. http://doi.org/10.1038/s41598-019-44588-3
26. Singh S, Zhang A, Dlouhy S, Bai S. Detection of large expansions in myotonic dystrophy type 1 using triplet primed PCR. *Front Genet*. 2014;5:94. http://doi.org/10.3389/fgene.2014.00094
27. Santoro M, Masciullo M, Silvestri G, Novelli G, Botta A. Myotonic dystrophy type 1: role of CCG, CTC and CGG interruptions within DMPK alleles in the pathogenesis and molecular diagnosis. *Clin Genet*. 2017;92(4):355-364. http://doi.org/10.1111/cge.12954
28. Peric S, Pesovic J, Savic-Pavicevic D, Rakocevic Stojanovic V, Meola G. Molecular and clinical implications of variant repeats in myotonic dystrophy type 1. *Int J Mol Sci*. 2021;23(1):354. http://doi.org/10.3390/ijms23010354
29. Tomé S, Dandelot E, Dogan C, et al. Unusual association of a unique CAG interruption in 5′ of DM1 CTG repeats with intergenerational contractions and low somatic mosaicism. *Hum Mutat*. 2018;39(7):970-982. http://doi.org/10.1002/humu.23531
30. Wenninger S, Cumming SA, Gutschmidt K, et al. Associations between variant repeat interruptions and clinical outcomes in myotonic dystrophy type 1. *Neurol Genet*. 2021;7(2):e572. http://doi.org/10.1212/NXG.0000000000000572
31. Miller JN, van der Plas E, Hamilton M, et al. Variant repeats within the DMPK CTG expansion protect function in myotonic dystrophy type 1. *Neurol Genet*. 2020;6(5):e504. http://doi.org/10.1212/NXG.0000000000000504
32. Cumming SA, Hamilton MJ, Robb Y, et al. De novo repeat interruptions are associated with reduced somatic instability and mild or absent clinical features in myotonic dystrophy type 1. *Eur J Hum Genet*. 2018;26(11):1635-1647. http://doi.org/10.1038/s41431-018-0156-9
33. Pešović J, Perić S, Brkušanin M, Brajušković G, Rakočević-Stojanović V, Savić-Pavićević D. Repeat interruptions modify age at onset in myotonic dystrophy type 1 by stabilizing DMPK expansions in somatic cells. *Front Genet*. 2018;9:601. http://doi.org/10.3389/fgene.2018.00601
34. Johnson NE. Myotonic muscular dystrophies. *Continuum (Minneap Minn)*. 2019;25(6):1682-1695. http://doi.org/10.1212/CON.0000000000000793
35. ACMG technical standards for clinical genetics laboratories (2021 revision). American College of Medical Genetics and Genomics. Accessed August 21, 2023. https://www.acmg.net/ACMG/Medical-Genetics-Practice-Resources/Genetics_Lab_Standards/ACMG/Medical-Genetics-Practice-Resources/Genetics_Lab_Standards.aspx?hkey=0e473683-3910-420c-9efb-958707c59589
36. New nomenclature and DNA testing guidelines for myotonic dystrophy type 1 (DM1). The international myotonic dystrophy consortium (IDMC). *Neurology*. 2000;54(6):1218-1221. http://doi.org/10.1212/wnl.54.6.1218
37. Kalman L, Tarleton J, Hitch M, et al. Development of a genomic DNA reference material panel for myotonic dystrophy type 1 (DM1) genetic testing. *J Mol Diagn*. 2013;15(4):518-525. http://doi.org/10.1016/j.jmoldx.2013.03.008
38. Buxton J, Shelbourne P, Davies J, et al. Detection of an unstable fragment of DNA specific to individuals with myotonic dystrophy. *Nature*. 1992;355(6360):547-548. http://doi.org/10.1038/355547a0
39. Shelbourne P, Davies J, Buxton J, et al. Direct diagnosis of myotonic dystrophy with a disease-specific DNA marker. *N Engl J Med*. 1993;328(7):471-475. http://doi.org/10.1056/NEJM199302183280704
40. Petronis A, Heng HH, Tatuch Y, et al. Direct detection of expanded trinucleotide repeats using PCR and DNA hybridization techniques. *Am J Med Genet*. 1996;67(1):85-91. http://doi.org/10.1002/(SICI)1096-8628(19960216)67:1<85::AID-AJMG15>3.0.CO;2-L
41. Guida M, Marger RS, Papp AC, et al. A molecular protocol for diagnosing myotonic dystrophy. *Clin Chem*. 1995;41(1):69-72. http://doi.org/10.1093/clinchem/41.1.69
42. Warner JP, Barron LH, Goudie D, et al. A general method for the detection of large CAG repeat expansions by fluorescent PCR. *J Med Genet*. 1996;33(12):1022-1026. http://doi.org/10.1136/jmg.33.12.1022
43. Lian M, Rajan-Babu IS, Singh K, Lee CG, Law HY, Chong SS. Efficient and highly sensitive screen for myotonic dystrophy type 1 using a one-step triplet-primed PCR and melting curve assay. *J Mol Diagn*. 2015;17(2):128-135. http://doi.org/10.1016/j.jmoldx.2014.10.001

44. Addis M, Serrenti M, Meloni C, Cau M, Melis MA. Triplet-primed PCR is more sensitive than Southern blotting-long PCR for the diagnosis of myotonic dystrophy type1. *Genet Test Mol Biomarkers*. 2012;16(12):1428-1431. http://doi.org/10.1089/gtmb.2012.0218
45. Kamsteeg EJ, Kress W, Catalli C, et al. Best practice guidelines and recommendations on the molecular diagnosis of myotonic dystrophy types 1 and 2. *Eur J Hum Genet*. 2012;20(12):1203-1208. http://doi.org/10.1038/ejhg.2012.108
46. Dryland PA, Doherty E, Love JM, Love DR. Simple repeat-primed PCR analysis of the myotonic dystrophy type 1 gene in a clinical diagnostics environment. *J Neurodegener Dis*. 2013;2013:857564. http://doi.org/10.1155/2013/857564
47. Mangin A, de Pontual L, Tsai YC, et al. Robust detection of somatic mosaicism and repeat interruptions by long-read targeted sequencing in myotonic dystrophy type 1. *Int J Mol Sci*. 2021;22(5):2616. http://doi.org/10.3390/ijms22052616
48. Gomes-Pereira M, Bidichandani SI, Monckton DG. Analysis of unstable triplet repeats using small-pool polymerase chain reaction. *Methods Mol Biol*. 2004;277:61-76. http://doi.org/10.1385/1-59259-804-8:061
49. Morales F, Vásquez M, Corrales E, et al. Longitudinal increases in somatic mosaicism of the expanded CTG repeat in myotonic dystrophy type 1 are associated with variation in age-at-onset. *Hum Mol Genet*. 2020;29(15):2496-2507. http://doi.org/10.1093/hmg/ddaa123
50. Morales F, Couto JM, Higham CF, et al. Somatic instability of the expanded CTG triplet repeat in myotonic dystrophy type 1 is a heritable quantitative trait and modifier of disease severity. *Hum Mol Genet*. 2012;21(16):3558-3567. http://doi.org/10.1093/hmg/dds185
51. Cumming SA, Jimenez-Moreno C, Okkersen K, et al. Genetic determinants of disease severity in the myotonic dystrophy type 1 OPTIMISTIC cohort. *Neurology*. 2019;93(10):e995-e1009. http://doi.org/10.1212/WNL.0000000000008056
52. Monckton DG, Wong LJ, Ashizawa T, Caskey CT. Somatic mosaicism, germline expansions, germline reversions and intergenerational reductions in myotonic dystrophy males: small pool PCR analyses. *Hum Mol Genet*. 1995;4(1):1-8. http://doi.org/10.1093/hmg/4.1.1
53. Morales F, Vásquez M, Santamaría C, Cuenca P, Corrales E, Monckton DG. A polymorphism in the MSH3 mismatch repair gene is associated with the levels of somatic instability of the expanded CTG repeat in the blood DNA of myotonic dystrophy type 1 patients. *DNA Repair (Amst)*. 2016;40:57-66. http://doi.org/10.1016/j.dnarep.2016.01.001
54. Overend G, Légaré C, Mathieu J, Bouchard L, Gagnon C, Monckton DG. Allele length of the DMPK CTG repeat is a predictor of progressive myotonic dystrophy type 1 phenotypes. *Hum Mol Genet*. 2019;28(13):2245-2254. http://doi.org/10.1093/hmg/ddz055
55. den Dunnen JT, Dalgleish R, Maglott DR, et al. HGVS recommendations for the description of sequence variants: 2016 update. *Hum Mutat*. 2016;37(6):564-569. http://doi.org/10.1002/humu.22981