



Association for Molecular Pathology
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TO: American College of Medical Genetics

FROM: Association for Molecular Pathology
Jan Nowak, MD, PhD, President

Clinical Practice Committee
Iris Schrijver, MD, Chair (ISchrijver@stanfordmed.org)

RE: *Comments on ACMG's Technical Standards and Guidelines for Myotonic Dystrophy Type 1 Testing*

The author should be commended as he put quite a bit of thought into this document.

Below we have listed some comments.

Please use HGVS nomenclature throughout text. While physicians and laboratorians use the CTG repeat number for interpretation, ACMG should have the official HGVS nomenclature for the CTG repeats in the document.

Line 13: Add space between “**TYPE 1(DM1)**”

Line 15 and throughout text: Please italicize the gene names

Line 18: Include “congenital” in addition to adult-onset multisystem disorder in defining the clinical description. i.e., The disease is an *adult/congenital*-onset multisystem disorder

Line 20: In the constellation of clinical findings, include “*respiratory insufficiency, hypogonadism and endocrine disturbances*” along with other multisystem features of this disorder. i.e.,characterized by progressive muscle weakness, myotonia, intellectual impairment, cataracts, cardiac arrhythmias, *respiratory insufficiency, hypogonadism and endocrine disturbances*.

This will complete the broad clinical spectrum of myotonic dystrophy to include both its forms (adult-onset and congenital) in a generalized manner.

Although the presence of a rare severe congenital form is described in lines 22-24, the initial clinical description of this disorder should not be restricted to the adult-onset form.

Therefore, following its initial clinical description in lines 18-22, the suggestion is to modify line 22 as follows:

The rare severe congenital form of the disorder results in mental.....respiratory complications. Those that survive.....www.geneclinics.org.

Line 30: Section 2.4 Mode of inheritance: Include *variable expressivity* along with variable penetrance. i.e., Mode of inheritance: Inheritance is autosomal dominant, with variable penetrance and *variable expressivity*.

Line 34-35: The word “*isoforms*” is missing. i.e., There is differential expression of the alternatively spliced *isoforms* in different tissues.

Line 35: It may sound better to say “A CTG repeat occurs in the 3’ untranslated portion of the last exon.” Rather than “The CTG repeat occurs in the 3’ untranslated portion of the last exon.”

Also:

Line 35: Replace the word “portion” to “region” in order to be consistent across the document. The author means to say that the last exon (exon 15) is non-coding and contains the 3’-UTR portion of the gene. i.e., The CTG repeat occurs in the 3’ untranslated *region corresponding* to the last exon.

Line 39 – Please define UTR as this is the first time that you use this abbreviation.

Line 46: Section 2.7 Genotype/Phenotype association: Include *mild myotonia* in addition to cataracts in the description of mildly affected patients. i.e., Mildly affected patients have 50 to 100 repeats, and these patients may only report cataracts and/or *mild myotonia*.

Line 47: When first introducing the concept of somatic mosaicism”, define it further as follows. i.e., *However, there is dynamic somatic mosaicism of the CTG repeat biased towards continuous expansion throughout the lifetime of an affected individual. Furthermore, there is an overlap in repeat sizes in patients with varying severity of the disease.*

Do not include the genetic counseling statement here and move it to the end of line 59 as a summary item. i.e., Following the description of the concepts of somatic mosaicism, overlap in repeat ranges, anticipation, influence of gender of the affected parent, spontaneous reversions, and non-penetrance in lines leading up to line 60, include the challenges in counseling as follows:

“ Therefore, challenges that call for caution in genetic counseling of patients/families affected by DM1 include, extreme clinical variability, somatic mosaicism, anticipation, influence of gender of the affected parent, and non-penetrance due to reversions”.

Line 54 – at time you use DM1 and other times you use DM as an abbreviation. Please be consistent.

Line 55: Consider replacing the word “correction” by “reversion”.

Line 56: In describing the mechanism of DM1 reverse mutations include the second mechanistic possibility that has been described. i.e., *A gene conversion mechanism, whereby the normal*

parental allele replaces the expanded allele, or a meiotic double recombination event leading to a disruption of the CTG repeats have been proposed as possible mechanisms for spontaneous contractions.

Reference: J. Med Genet, 2001 38:850-852.

Line 61-62: Consider adding the phrase “protein coding portion” i.e., Since the repeat is not in the protein coding portion, (i.e., the exons), the molecular mechanism.....to explain.

Line 68 - Please italicize the gene names

Line 74: Ethnic association of the DM1 mutation: Include a range of prevalence.

i.e., The prevalence of DM1 ranges from 1/100,000 in some areas of Japan to 1/10,000 in Iceland, 1/8000 amongst “Caucasians” (replace “whites” by “Caucasians”), 1/18,000 amongst Asians and a very low reported incidence amongst African blacks. The estimated world wide prevalence of DM1 is 1/20,000 (www.genetests.org)

Also:

Line 74 – Whites or Caucasians?

Line 78-82: Use the terms “analytical sensitivity” (i.e., proportion of positive tests in genotype is present) and “analytical specificity” (i.e., the proportion of negative tests if the genotype is not present) to be distinguishable from “clinical sensitivity” (proportion of positive tests if DM1 is present) and “clinical specificity” (proportion of negative tests if DM1 is not present)

Line 78: Therefore, the analytical sensitivity and specificity of tests that effectively detect and measure the CTG-repeats in the 3’ UTR of the DMPK gene approaches 100%.

NOTE: One must be wary of stating that the analytical sensitivity “is” 100% (stated in the draft version, see line 79) since rare cases of a large expansion with somatic mosaicism “may” not be detectable.

Line 81-82: However, the DM1 mutation.....50 to 80 repeats. 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 one or both alleles in the range of 50 to 80 CTG repeats. For CTG repeats larger than this range, the clinical sensitivity and specificity are high and expected to approach 100%.

Line 86 - Please italicize the gene names

Also:

Line 86: Include the mode of inheritance for DM2: autosomal dominant.

Line 88: In describing the similarities of DM2 with DM1, include hypogonadism and cataracts to the mix. i.e., *myotonia, cardiac involvement, insulin sensitivity, hypogonadism, cataracts, and histological abnormalities in skeletal muscle.*

Line 93: Section 3.2 Indications for Genetic testing: Include additional indications for testing to include the following:

1. *Symptomatic confirmatory diagnostic testing*
2. *Predictive testing following the identification of mutation in an affected family member*
3. *Prenatal diagnosis for at risk pregnancies following ultrasonographic evidence to include fetal hypotonia, reduced fetal movements, positional abnormalities and/or polyhydramnios.*

Line 93 - To be consistent, please change “Genetic” to lower case in “Indications for Genetic testing”

Line 94-98: “Onset in adulthoodobserved on ultrasound”. *Consider removing these sentences if all indications of testing have already been laid out as described above*

Line 130 and throughout text– Southern Blotting/blots is laboratory slang, please revise.

Line 165 – blotted is laboratory slang, please revise.

Line 173: Decreasing the electrophoretic.....somatic mosaic expansions. Consider adding the outcome of this modification. i.e., *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*

Line 183-184: Are you really quantitating or sizing? Please revise “...and post-PCR analyses be optimized to ensure for the accurate and unambiguous quantitation of repeat length.”

Line 194 - Are you really quantitating or sizing? Please revise “Accurate quantitation of repeat sizes should be empirically determined by comparison to appropriate external or internal standards.”

Line 208 – Why do you need the primers in the report? That is not required for any other assay and might be considered proprietary. Please remove “If PCR methodology was used then a description of the primer pair(s) should be included”

Line 225 – Need statement on report about a laboratory developed test (LDT). Please confirm with someone from the FDA regarding this statement...“This test was developed and its performance characteristics determined by this laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration. *The FDA 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.”

Figure 1 – note there is no ladder in your figure, so it does not match the recommendation in the document. Please use another figure.

Line 235: 4.3.3.2 the word “uncertainty” has been spelt incorrectly.

Part B: General comments to be considered for inclusion in the indications for testing

A recent article describing a 13 year experience following prenatal diagnosis of DM1 included some recommendations for prenatal diagnosis. These are summarized below and may be considered for inclusion into the final guidelines:

Reference: Martorell, L. et al. PRENATAL DIAGNOSIS 2007, 27: 68-72.

1. Suggestion to offer prenatal diagnosis to a DM1 family regardless of maternal disease involvement. The authors state that the maternal germline distribution of expanded alleles, rather than their clinical involvement is the most critical factor involved in their risk of conceiving a CMD (congenital myotonic dystrophy) child.
2. Molecular confirmation of DM1 on BOTH parents must always be performed before requesting a prenatal diagnosis to avoid the possibility of misinterpretation of results. In the author's opinion, this allows the parental origin of the DM1 allele to be taken into account, owing to the unpredictable behavior of permutation alleles during intergenerational transmissions.

Section 4.2 Methodological considerations

Since this is a guideline statement, it would be useful to state clearly whether or not both PCR and Southern blots must always be performed on all cases irrespective of PCR results or should Southern analysis be used as a reflex test for single allele PCR results. In section 4.2.2.3 it is stated that "when two normal alleles are identified, the DM1 diagnosis can be excluded" Can this be interpreted by a development laboratory as not requiring Southern analysis? If so, it would be useful to state this fact as a guideline.

Section 4.2.1. Southern blots

Consider including a statement regarding "whether or not" the Alu repeat polymorphism when seen on Southern blots needs to be confirmed with an alternate restriction enzyme in order to be considered as a non-pathological finding in the final result interpretation.

Line 166: Increasing electrophoretic migrationsmaller expansions. Does this mean increasing velocity? This statement seems vague as several factors affect migration, a higher % agarose gel will give better resolution of lower MW fragments, high voltage, gel thickness, buffer volume are other factors. So the author may consider a more inclusive but generalized statement to state the fact being described

Section 4.3 Interpretations

Line 228: "Proximal myotonic by this test". The usage of this statement on result reports must be restricted based upon **at-least** the age of the patient being tested. This statement would

not be applicable for inclusion on prenatal test reports, and children being tested for a possible diagnosis.

Consider adding the following statement on reports with a homozygous PCR allele:

CTG repeat > 100 repeats (or the upper limit as determined by the laboratory) cannot be reliably measured by PCR analysis. In these cases repeat size is determined by Southern blot analysis.

We appreciate the opportunity to comment on this important document.