EMQN Best Practice Guidelines and Recommendations on Myotonic Dystrophy types 1 and 2

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Abstract

Myotonic dystrophy is a clinically and genetically heterogeneous disorder. Myotonic dystrophy type l (DM1) and type 2 (DM2) are both caused by unstable DNA sequences comprising repetitive elements in untranslated regions of a gene: A [CTG]n trinucleotide repeat sequence in the 3' region of the *DMPK* gene located at chromosome 19ql3 (DM1), and a [CCTG]n tetranucleotide repeat in the first intron of the *CNBP* (*ZNF9)* gene located at chromosome 3q21 (DM2). The clinical features of DM are caused by a mechanism in which mRNAs containing expanded CUG and CCUG repeats alter normal nuclear functions, including the function of the mRNA splicing machinery. In turn this leads to alternative transcripts in (downstream-regulated) muscle expressed genes. Southern analysis and/or PCR-based approaches allow the detection of expanded sequences in myotonic dystrophy types 1 and 2 in virtually all cases. Here, we describe Best Practice Guidelines for the clinical molecular genetic analysis in the myotonic dystrophies type 1 and 2, including presymptomatic and prenatal testing.

Molecular genetic defects in myotonic dystrophy types 1 and 2

Myotonic dystrophy (DM) is an autosomal dominant, multisystem disorder. Myotonic dystrophy is clinically heterogeneous, and at the molecular level at least two types can be distinguished: DM type 1 (DM1; Steinert disease), and DM type 2 (DM2; proximal myotonic myopathy (PROMM), or Ricker syndrome). DM1 is the most common form of muscular dystrophy in adults with an estimated incidence of 1:8000 (Harper, 2001). Although reliable data are not available, the incidence for DM2 seems to be much lower compared to DM1, is population dependent, reaching a peak incidence in Germany as high as DM1. Two different mutations are responsible for DM: DM1 (OMIM #160900) is caused by a $[CTG]_n$ repeat expansion in the 3'-untranslated region of the *DMPK* gene located within chromosome band 19ql3.3 (Brook et al., 1992; Fu et al., 1992; Mahadevan et al., 1992), while DM2 (OMIM #602688) is caused by a large $[CCTG]_n$ repeat expansion in intron l of the *CNBP* gene, at chromosome 3q21 (Liquori et al., 2001; Ricker et al., 1999; Ranum et al., 1998).

The number of CTG-repeat units in a stable nonpathogenic *DMPK* allele ranges between 5 and 36. *DMPK* alleles containing over 36 CTG-repeat units demonstrate a length-dependant risk of instability on transmission. Alleles containing a CTG-repeat with a length of 51-150 may be either asymptomatic or may give rise to minimal or classical DM1. A more severe DM1 phenotype is associated with *DMPK* alleles with sizes > 150 CTG-repeat units. An overview of the phenotype-genotype correlation (The International Myotonic Dystrophy Consortium, 2000; Hamshere et al., 1999) is provided in Table 1. Thus, the severity of the disease and the age of onset is correlated with the number of CTG-repeat units within the expanded DNA sequence, although the individual variability is high (Lavedan et al., 1993; Hamshere et al., 1999); Ashizawa et al., 1992; Harley et al., 1993; Gennarelli et al., 1996). An increasing number of CTG-repeat units within the *DMPK* gene in successive generations in a family is associated with an earlier onset of the disorder. This phenomenon is known as "anticipation" (Howeler et al., 1989). The DM1 parental sex plays an important role in determining the level of intergenerational instability. Severe expansions are transmitted almost exclusively through female meioses, explaining why congenital DM1 is virtually exclusively transmitted by the mother.

The *ZNF9* gene harbors a complex repeat $(TG)_n(TCTG)_n(CCTG)_n$, and expansions of the CCTG-repeat array within this complex repeat are the cause of DM2 (Liquori et al., 2001). Nonpathogenic alleles contain up to 26 CCTG repeat units. In DM2 patients, the range of repeat units is extremely wide, ranging from 75 to over 11,000 units, with a mean of 5,000 (Liquori et al., 2001). The CCTG-repeat tract in affected children is often smaller compared to their affected parent, a phenomenon called "contraction". However, the age-related somatic variability that characterizes each patient complicates the interpretation of these data (Liquori et al., 2001). Unlike in DM1, there is no significant association between the age of onset in DM2 and the number of CCTG-repeat units (Ranum and Day, 2002).

It has been demonstrated that in both DM1 and DM2 CUG- and CCUG-containing transcripts accumulate in ribonuclear foci in cells (Lavedan et al., 1993), altering the regulation and localization of the CUG-binding protein (CUG-BP) (Timchenko et al., 1996) and three different forms of the RNA-binding protein Muscleblind-like 1 (MBNL1) (Miller et al., 2000; Fardaei et al., 2002). Both proteins play a role in affecting alternative splicing through antagonistic effects, in which CUG-BP promotes the inclusion of exons that are normally expressed during fetal development, whereas MBNL1 favors the adult splicing isoform (Ho et al., 2005). Changes in the regulation of these proteins can cause aberrant splicing of genes expressed in DM target tissues. Dysregulation of the insulin receptor (IR), chloride channel 1 (CLCNl), skeletal ryanodine receptor (RYRl) and the sarcoplasmic reticulum Ca2+-ATPase (SERCA) mRNAs has been observed in muscle cells from DM patients, confirming the hypothesis of a pathogenic role for the expanded DMPK and CNBP mRNAs (Kimura et al., 2005; Savkur et al., 2004; Savkur et al., 2001; Mankodi et al., 2002; Charlet et al., 2002).

Table 1 - Size ranges of CTG repeats in the *DMPK* gene and corresponding interpretation

Number of repeats	Stability of repeat	DM-phenotype
5 - 36 (normal range)	stable	no DM
37 - 50	may be unstable	no DM
51 - 150	unstable	no, minimal, or classical DM
>150	unstable	classical, juvenile, or congenital DM

Clinical symptoms and indications for molecular genetic analysis

Both DM1 and DM2 are multisystem disorders, sharing comparable core diagnostic criteria and multi-organ involvement (Harper, 2001; Harper, 2004). There are, however, specific clinical aspects for each type.

Myotonic dystrophy type 1

There is a wide range of symptoms in DM1, ranging from mild features, such as baldness and cataracts, to very severe features including muscle weakness that may involve the heart. Many of the severely affected patients die of cardiac failure or respiratory distress. The cardiac involvement in DM1 may range between asymptomatic ECG abnormalities and sudden death. A severe abnormality on the ECG and a diagnosis of an atrial tachyarrhythmia predict sudden death (Groh et al. 2008).

From a clinical perspective DM l may be classified into four different subtypes,: (i) mild; (ii) classical; (iii) juvenile and (iv) congenital types.

Mild DM1: This is typical of mildly symptomatic patients in whom premature cataracts and baldness may be the only clinical features. A late-onset myopathy may develop and myotonia may only be detectable on electromyography.

Classical or adult-onset DM1: The age of onset is typically in the 2nd decade of life. The most frequent symptoms are distal weakness, involving the long finger flexors of the arms and the dorsiflexors of the legs, leading to symptoms relating to the strength of hand grasping and higher frequencies of stumbling. In addition to cataracts and baldness as in minimal DM1, clinical myotonia, gastro-intestinal symptoms and fatigue may occur.

Juvenile DM1: This form resembles the classical form of myotonic dystrophy. However, it is more clearly associated with cognitive and behavioral abnormalities, e.g. difficulties in learning and socialization at school. Muscle involvement may be minimal in the juvenile presentation.

Congenital DM1. Polyhydramnios and poor fetal movements precede the birth of an infant with congenital DM1. The affected parent is virtually always the mother and congenital DM1 occurs in a quarter of offspring of affected DM1 mothers (Harper,

2001). The infant is typically a floppy baby with failure to thrive and severe respiratory distress. Facial and jaw muscles are weak and produce a tented upper lip with difficulties in feeding and suckling. Clubfeet and joint contractures are also common. Myotonia is typically absent clinically, and may be difficult to detect initially even on EMG. Mental retardation and developmental delay are common, and a high mortality rate exists in the perinatal period. Muscle biopsies of patients with congenital DM1 may reveal only variability of the size of the fibers. However, none of the characteristics found in muscle biopsies of patients with classical or adult-onset DM1 myotonic dystrophy are present. Therefore, after clinical suspicion of congenital DM1, a diagnosis can only be established by DNA analysis.

Myotonic dystrophy type 2

The DM2 phenotype shows similarities to that of DM1 (myotonia, cataract, muscle weakness), but also differences (pain sensation not known in DM1, but less cardiac and brain involvements and no congenital form of the disease). DM2 patients may present with a DM1-like phenotype but with a predominantly proximal involvement, as typified in the original clinical description of the disorder (Day et al., 1999), or with a more severe proximal muscle involvement with remarkable muscle wasting without clinical myotonia (Udd et al., 1997; Rotondo et al., 2005). The characteristic clinical presentation of typical DM2 is that of predominantly proximal muscle weakness, with muscle pain, but no atrophy (Proximal myotonic myopathy (PROMM) phenotype). Myotonia is variable (both clinically as well as on EMG). However, in its mildest form, DM2 can be difficult to recognize. No congenital form has been described. The mean DM2 onset is in the 3rd and 4th decades of life.

In summary, the clinical challenge in DM1 lies in supplying optimal care for this multisystem disease whereas in DM2 it is a diagnostic challenge to recognize the disease. Detection of a repeat expansion in the *DMPK* gene and the *CNBP* gene is fast, inexpensive, and reliable. Both the clinical sensitivity and the clinical specificity are >99% (Eurogentest clinical utility gene cards). In a situation like this, no restricting clinical criteria before undertaking DNA analysis are warranted.

Molecular diagnostic analysis in DM1 and DM2

The first step in the molecular analysis of DM1 or DM2 is to analyze whether an individual has two alleles with a low number of repeats that can easily be detected by conventional PCR and fragment-length analysis. If only one allele-size is detected, subsequent techniques such as repeat-primed PCR and or Southern blotting is used to detect possible repeat expansions. In prenatal DM1 testing, analysis of DNA from the mother is also required to exclude maternal contamination in the fetal samples. The combination of these data allows interpretation and reporting. The delivery of diagnostic molecular genetics services for pre/post-natal DM (outlined in the flow chart in Fig. 1) requires in-house validation, on-going quality control processes and the supervision by a credentialed clinical molecular geneticist.

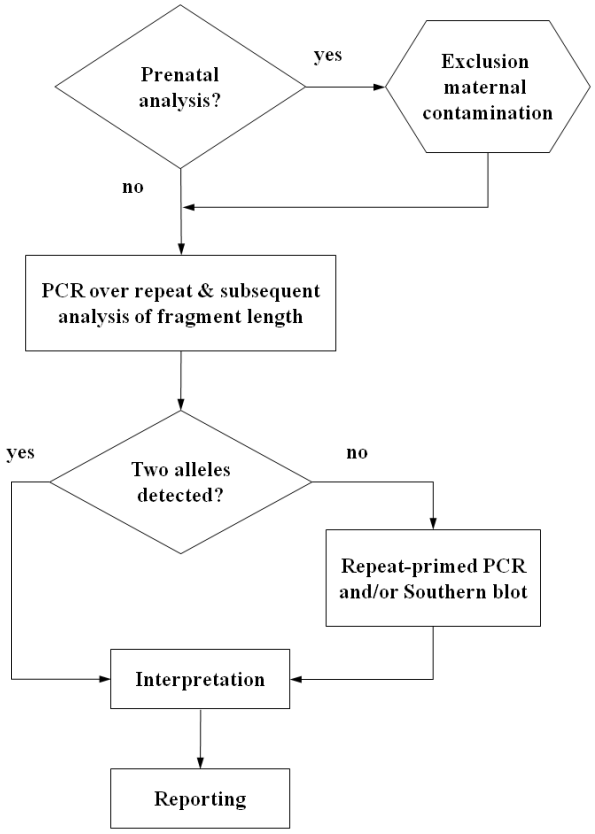

Figure 1 - Flow-chart for molecular DM1 and DM2 analysis

Molecular diagnostic tests in DM1

PCR and fragment-length analysis

In the lower range of DM1 expansions, the best analytical method is PCR. Alleles containing between 5 and 100 to 125 CTG-repeat units can be detected and characterized using synthetic fluorescently labeled primers flanking the CTG-repeat region, followed by analyzing the length of the amplified products directly by capillary electrophoresis. Larger expansion can be detected either by Triplet-repeat Primed PCR or Southern blotting of genomic DNA or Long-Range PCR products. Triplet-repeat Primed PCR and Southern blotting of Long-Range PCR products are sensitive and reliable techniques to analyze the presence of expansions. However, for sizing of the large DM1 repeat expansions (CTG>200), which has little or no clinical consequences, Southern blotting of genomic DNA would be the method of choice.

Triplet-repeat Primed (TP)-PCR

The TP-PCR is a PCR with three primers, where one lies outside the repeat, one within the repeat (which is added in limiting amounts) that also has a sequence-tail complementary to the third, universal, and fluorescently-labeled primer (Warner et al., 1996). This will result in a mixture of PCR-fragments of different sizes that can be analyzed by capillary electrophoresis. In theory, the TP-PCR will detect expansions independent of their lengths, and, although expansions in all size ranges can be detected, no reliable information about the length of the expanded repeat will be obtained. In DM1 expansion analysis (see Fig. 2 for an example), the TP-PCR has proven to be an accurate technique, with no false positive or negative results (Falk et al., 2006).

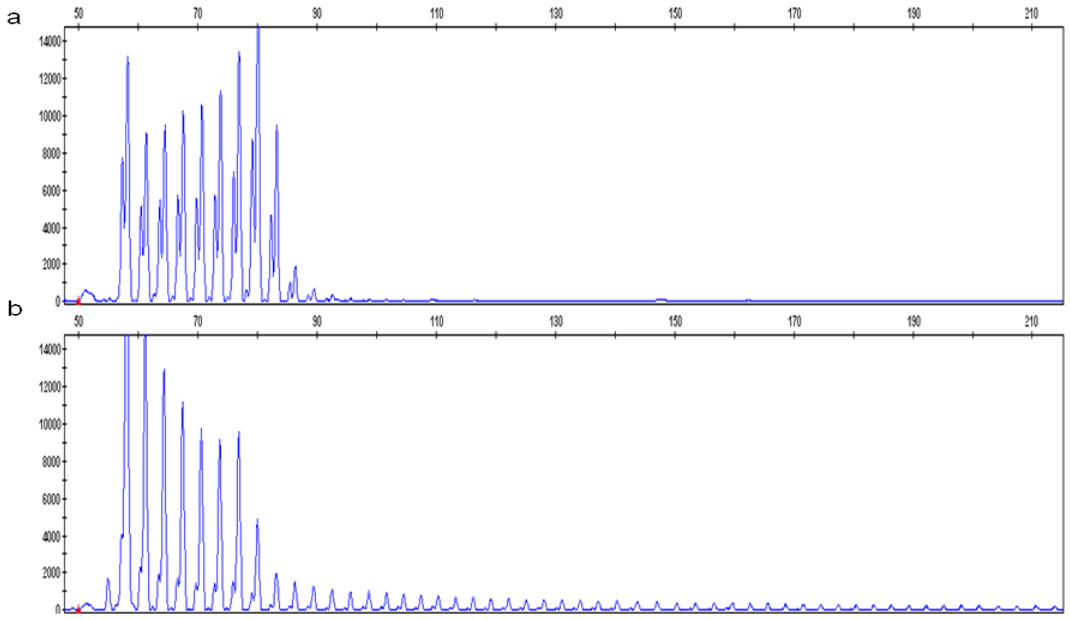

Figure 2 - Fragment length analysis of TP-PCR products of the CTG repeat in the *DMPK* gene

Fluorescently labeled PCR products of **a**) a healthy individual having two normal alleles (5 and 13 CTG repeats) and **b**) an affected individual with one normal allele (5 repeats) and one expanded allele (> 50 repeats).

Southern blotting of long-range PCR-products

This Southern blot method (for an example, see Fig. 3) requires precise PCR conditions to ensure the amplification of larger fragments, monitored by using appropriate positive and negative (healthy) controls, followed by probing with an end-labeled $[CTG]_{5or10}$ probe (Gennarelli et al., 1998).. Due to somatic heterogeneity, expanded DM1 alleles will in virtually all cases appear as smears or multiple fragments on Southern blots, irrespective of the used material (genomic or long-range PCR products).

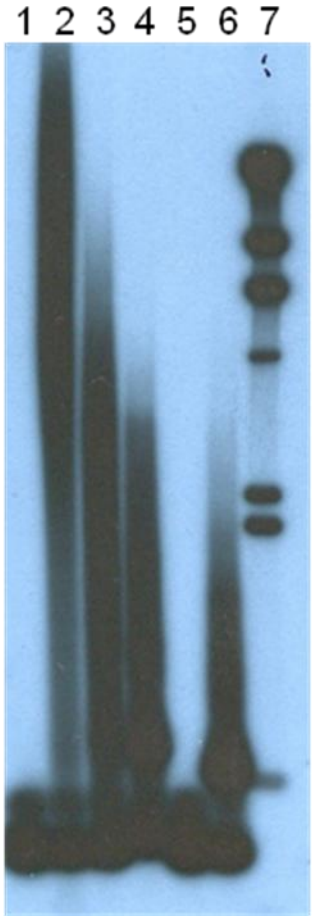

Figure 3 - Southern blotting of long-range PCR-products of the CTG repeat in the *DMPK* gene.

Long-range PCR fragments were subjected to electophoresis and capillary blotting to a nylon membrane. Subsequently, the membrane was hybridized with a ^{32}P-endlabelled $(CAG)_5$ probe. Visualised repeats were from healthy controls (lanes 1 and 5), and patients with one normal and one expanded allele of aproximately 1,500 (lane 2), 300 (lane 3), 150 (lane 4) and 120 (lane 6) repeats. Repeat lengths sized by the Lamda *Hind*III size marker (lane 7) and PCR fragment length analysis (not shown).

Southern blotting of genomic DNA

Southern blotting of genomic DNA (for an example, see Fig. 4), digested with an appropriate restriction enzyme (*Eco*RI, *Bam*HI, *Nco*I, or *Bgl*I), has been the gold standard for the detection of *DMPK* alleles containing 100 CTG-repeat units and over since the identification of the *DMPK* gene (Brook et al., 1992). Several different probes have been reported for hybridization (Brook et al., 1992; Fu et al., 1992; Buxton et al., 1992; Shelbourne et al., 1993). However, this procedure is time-consuming and has been replaced by TP-PCR and or by Southern blotting of long-range PCR-products in most diagnostic centers.

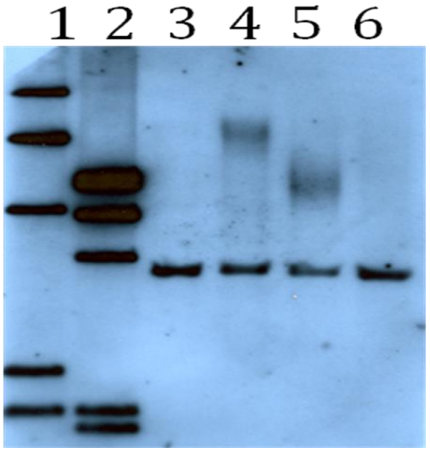

Figure 4 - Southern blotting of genomic DNA probed for the *DMPK* gene.

Genomic DNA samples were fragmented using *Bgl*I and subjected to electophoresis and capillary blotting to a nylon membrane. Subsequently, the membrane was hybridized with a ^{32}P-labelled probe for the *DMPK* gene. Visualised fragments were from healthy controls (lanes 3 and 6), and patients with one normal and one expanded allele (lanes 4 and 5). Estimation of the lengths of the repeat expansions can be done using the size markers (lanes 1 and 2).

Indirect DNA analysis

Though direct detection of expansions in the *DMPK* gene is the most straightforward technique, indirect DNA analysis is used in some cases. Polymorphic markers surrounding the *DMPK* gene that can be used for such an indirect analysis are outlined in Table 2.

Table 2 – Markers flanking the DMPK gene

Marker	***Location in bp (Human RefMap)***	***Position in cM (Genethon)***	***Allele Size (bp)***
D19S217	*40,713,572 -40,713,794*	*67.8*	*219-233*
D19S574	*41,555,837-4,1556,038*	*69.20*	*164-202*
D19S918	*NA*	*69.50*	*140-182*
D19S219	*42,423,170-42,423,347*	*69.90*	*160-190*
DMPK	*42,701,722 -42,714,534*	*70.14*	
D19S902	*44,758,758 - 44,758,960*	*76.20*	*199-217*

Molecular diagnostic tests in DM2

Due to the extreme expansion sizes (to > 40 kb) that may be present in DM2 patients, it is important to ensure isolation of high molecular weight DNA from peripheral blood.

PCR and fragment-length analysis

In the lower range of DM2 repeat sizes, the best analytical method is PCR. Such alleles can be detected and characterized using synthetic, fluorescently-labeled primers flanking the complex-repeat region (also known as the CL3N58 repeat marker), followed by sizing of the amplified products directly by capillary electrophoresis. Due to the variability of the different units of this complex $(TG)_n(TCTG)_n(CCTG)_n$, repeat, the precise length of the pathogenic CCTG unit within this repeat can only be determined by sequencing. However, since the difference between normal alleles (repeats up to 26 CCTG units) and disease-associated alleles (75 units and more, with a mean of 5,000) almost always is evident, exact sizing is not routinely performed. Larger expansion can be detected either by Quadruplet-repeat Primed PCR or Southern blotting of genomic DNA or Long-Range PCR products. As in DM1 analysis, Southern blotting of genomic DNA would be the method of choice when sizing is required.

Quadruplet-repeat Primed (QP)-PCR

This technique, also known as tetraplet-repeat primed PCR, is similar to the TP-PCR used for DM1, but now with a primer in the tetra-nucleotide repeat (Day et al., 2003; see Fig. 5 for an example). In theory, the QP-PCR will detect expansions independent of their lengths, and, although expansions in all size ranges can be detected, no reliable information about the length of the expanded repeat will be obtained.

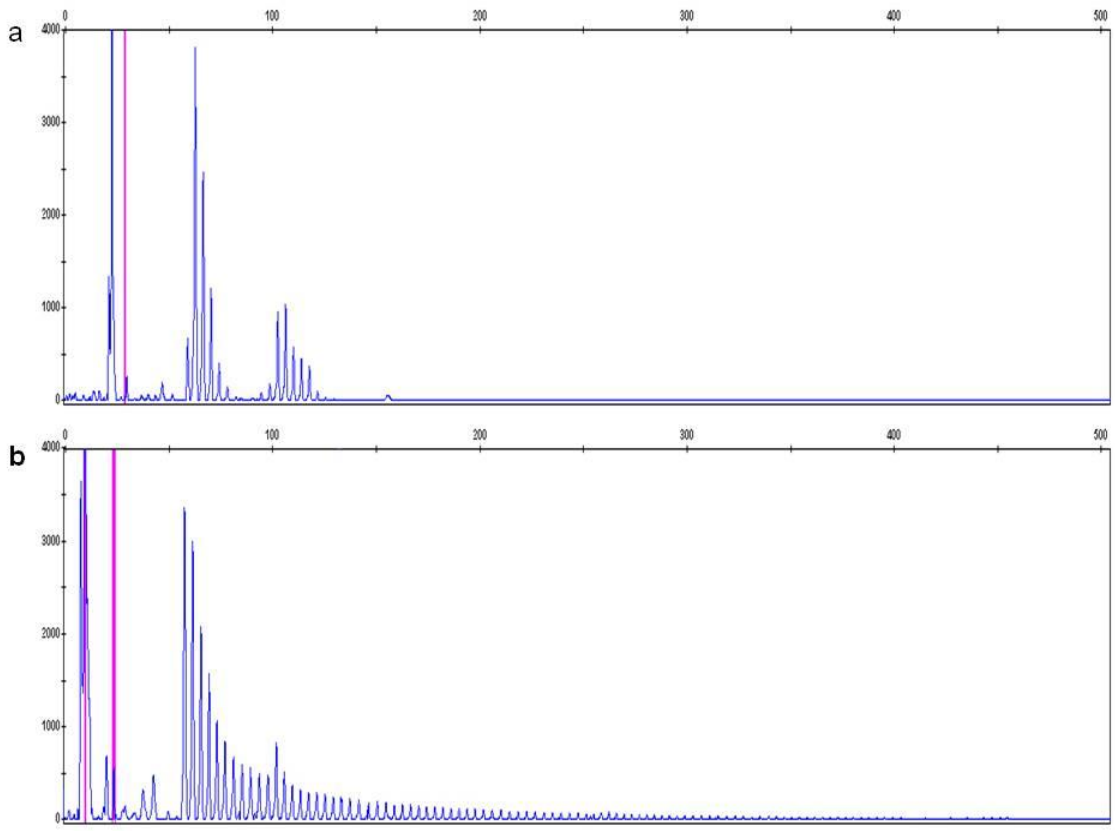

Figure 5 - Fragment length analysis of QP-PCR products of the CCTG repeat in the *CNBP* gene.

Fluorescently labeled PCR products of **a**) a healthy individual and **b**) an affected individual with one normal allele and one expanded allele (> 100 repeats).

Southern blotting of long-range PCR-products

This Southern blot method (see Fig. 6 for an example) requires precise PCR conditions to ensure the amplification of larger fragments, monitored by using appropriate positive and negative (healthy) controls. Southern blotting of these fragments is followed by probing with an end-labeled $[CCTG]_{5or6}$ probe (Schoser et al., 2004). Due to somatic heterogeneity, expanded alleles will in virtually all cases appear as smears on Southern blots, irrespective of the used material (genomic or long-range PCR products). Using this technique in DM2 diagnostics, one should be aware of false positives. Therefore, negative controls (healthy subjects) should be free of smears on the blot.

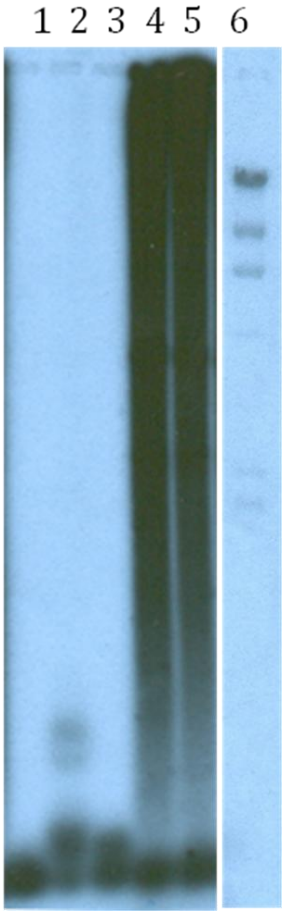

Figure 6 - Southern blotting of long-range PCR-products of the CCTG repeat in the *CNBP* gene.

Long-range PCR fragments were subjected to electophoresis and capillary blotting to a nylon membrane. Subsequently, the membrane was hybridized with a ^{32}P-end-labelled $(CAGG)_5$ probe. Visualised repeats were from healthy homozygous (lane 1) and heterozygous (lane 2 and 3) controls and patients with one normal and one expanded allele (lanes 4 and 5). Lane 7 shows the Lamda *Hind*III size marker.

Southern blotting of genomic DNA

Direct analysis by Southern blotting (for an example, see Fig. 7), after digestion of genomic DNA with *Eco*RI, *Taq* I, or alternative enzymes, followed by hybridization using the CL3N58 probe, was the first method used for studying the DM2 mutation (Liquori et al., 2001). Due to the extreme size of the expansion and the level of somatic instability as well as cross hybridizations of the probe, only a very heterogeneous smear can be visualized, limiting the sensitivity of this test to approximately 80% of known carriers when applied without additional precautions. Several improvements of the basic protocol can be adopted in order to minimize possible sources of false negative results, such as the inclusion of a reference probe to allow quantification of the non-expanded alleles (Jakubiczka et al., 2004). However, like in DM1 analysis, this procedure is time-consuming and has been replaced by QP-PCR or by Southern blotting of long-range PCR-products by most diagnostic laboratories.

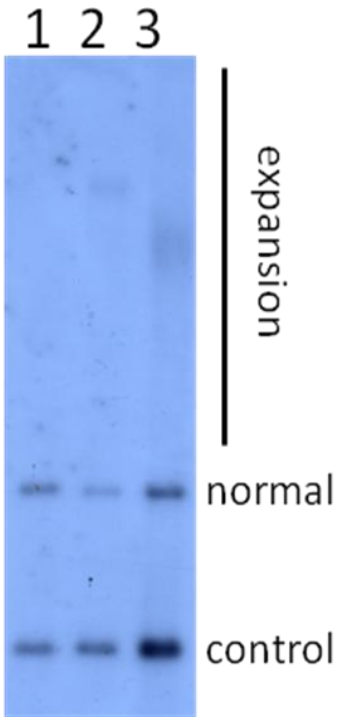

Figure 7 - Southern blotting of genomic DNA probed for the *CNBP* gene.

Genomic DNA samples were fragmented using *EcoR*I and subjected to electophoresis and capillary blotting to a nylon membrane. Subsequently, the membrane was hybridized with a mixture of the CL3N58 (within the *CNBP* gene) and a control probe (both ^{32}P-labelled). Visualised fragments were from a healthy control (lanes 1), and patients with one normal and one expanded allele (lanes 2 and 3). The signals from the control probe and the *CNBP* probe (normal and expansion) are indicated. Note the decrease in intensity of the normal allele in the patients with an expansion, when compared with the control probe signal.

Interpretation and Reporting

Myotonic dystrophy type 1

Interpretation

There is an association between CTG repeat size and the age of onset and severity of symptoms (see table 1). However, there is a large inter-individual variability and one should be careful in the prediction of the expected phenotype in individual cases (Lavedan et al., 1993; Hamshere et al., 1999); Ashizawa et al., 1992; Harley et al., 1993; Gennarelli et al., 1996). Therefore, the boundaries in repeat lengths of the four groups in Table 1 should not be interpreted too rigidly.

Reporting

In DM, it is sufficient to report the size range of a repeat rather than an exact repeat length, i.e. "a normal (CTG)n repeat length of ($5<n<37$)" or "a large expansion of the (CTG)n repeat ($n>150$)". Whenever an exact allele size is reported it should be accompanied by a statement of the testing laboratory's uncertainty of measurement in the sizing.

For the reporting of normal allele sizes (n = 5-36), which are determined by PCR and subsequent capillary electrophoresis, internal control standards from sequence-verified samples should be included. Alternatively, exact allele sizes can be obtained by DNA sequence analysis. For larger expansions, a range of repeat-lengths should be estimated based on comparisons with molecular weight standards and/or characterized control samples.

The result of the appropriate tests (see Fig. 1) will lead to one of the following possibilities and recommended reporting guidelines.

Two alleles in the normal range (either homozygous or compound heterozygous) This excludes the diagnosis of DM1; When it concerns a fetus, it is not affected with DM1.
One normal allele and one allele in the range of 37-50 repeats a) This excludes the diagnosis of DM1; when it concerns a fetus, it is not affected with DM1. b) Repeats of this length may or may not expand in future generations. c) Relatives (including offspring) of the counselee may be at risk of developing DM1. Therefore, they should be offered counseling. An offer of repeat-length analysis to those relatives is warranted.
One normal allele and one allele in the range of 51-150 repeats a) When symptoms are evident, the diagnosis of DM1 is confirmed b) When symptoms of DM1 are not evident (asymptomatic family member or fetus), the individual is at risk of developing DM1, although individuals with a repeat expansion of this size may also remain symptomless. c) Counselees in the reproductive age, and especially women, are at risk of having children with the congenital form of DM1. d) Relatives (including offspring) of the counselee may be at risk of developing DM1. Therefore, they should be offered counseling. An offer of repeat-length analysis to those relatives is warranted.
One normal allele and one allele with a repeat expansion of >150 a) The diagnosis of DM1 is confirmed; When it concerns a fetus, it is affected with DM1 and has a high risk to be more severely affected than the affected parent. b) Counselees in the reproductive age, and especially women, are at risk of having children with the congenital form of DM1. c) Relatives (including offspring) of the counselee may be at risk of developing DM1. Therefore, they should be offered counseling. An offer of repeat-length analysis to those relatives is warranted.

Myotonic dystrophy type 2

Interpretation

Since variability in the sizes of the different units of the complex repeat, $(TG)_n(TCTG)_n(CCTG)_n$, in the CNBP gene exists, the precise length of the pathogenic CCTG unit within this repeat can only be determined by DNA sequencing. However, since repeats up to 26 CCTG units are considered to nonpathogenic while disease-associated alleles contain 75 units and more, this is not routinely performed. Experiences from several laboratories suggest that most frequently encountered pathogenic repeats reside in the high range (with a mean of 5,000 repeats). Alleles between 26 and 75 CCTG units (grey area) have, as a consequence, not been described and, if present, not well characterized.

Reporting

If reporting allele sizes, estimates of the uncertainty of measurement in the sizing in the testing laboratory should be reported. For allele sizes determined by PCR and subsequent capillary electrophoresis, a deviation of 2 repeat units may be present. For (larger) expansion, a range of repeat-lengths should be estimated on basis of markers and/or control samples. However, in the reports of most laboratories the exact length of the repeat is not given. The result of the appropriate tests (see Fig. 1) will lead to one of the following possibilities and subsequent recommended reporting guidelines.

Two alleles within the normal range (up to 26 CCTG-repeat units) This excludes the diagnosis of DM2.
One normal allele and one allele with a repeat expansion of >75 CCTG-repeat units a) The diagnosis of DM2 is confirmed. b) Relatives (including offspring) of the counselee may be at risk of developing DM2. Therefore, they should be offered counseling. An offer of repeat-length analysis to those relatives is warranted.

Resources

Table 3 Primer sequences.
Abbreviations : LR: long-range, TP: triplet -repeat primed, QP: quadruplet-repeat primed, C: Copenhagen, N: Nijmegen, R: Rome, W: Würzburg. Repeat sequences are underlined. * These primers amplify the CL3N58 marker, which is defined as a CAGG repeat and is the reverse complement of the CCTG repeat in the CNBP gene. (a) A rare polymorphism affecting this base was detected on a CNBP allele that also harbors a CCTG expansion (E. Ormshaw, SW Thames Molecular Genetics Diagnostic Laboratory, London, personal communication).

Gene	Location	Purpose	Centre	Sequence (5'-3')
DMPK	Forward	PCR	C	cttcccaggcctgcagtttgcccatc
	Reverse	PCR	C	gaacggggctcgaagggtccttgtagc
	Forward	PCR	N	ctcgaagggtccttgtagcc
	Forward	TP-PCR	N	gaagggtccttgtagccgggaa
	Forward	LR-PCR	N	ccgttggaagactgagtg
	Reverse	PCR	N	tgcacaagaaagctttgcac
	Reverse	TP-PCR	N	tacgcatcccagtttgagacgcagcagcagcagcag
	Universal	TP-PCR	N	tacgcatcccagtttgagacg
	Reverse	LR-PCR	N	ctggccgaaagaaagaaatg
CNBP	Forward	PCR	W	ggccttataaccatgcaaatg*
(ZNF9)	Forward	PCR	N	ttggacttggaatgagtgaatg
	Forward	LR-PCR	N/ W	ggccttataaccatgcaaatg*
	Reverse	(LR)-PCR/QP-PCR	W/ R	gcctaggggacaaagtgag(a)*
	Reverse	PCR	N	agccgagatcataccactgc
	Reverse	LR-PCR/QP-PCR	N	agcctaggggacaaagtgag
	Forward	QP-PCR	N/ W/ R	tacgcatcccagtttgagacgcctgcctgcctgcctgcctg
	Universal	QP-PCR	R	tacgcatcccagtttgagacg

Websites:

Genereviews DM1:

http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=myotonic-d

Genereviews DM2:

http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=myotonic-d2

Eurogentest clinical utility gene cards:

http://www.eurogentest.org/web/info/public/unit3/geneCards.xhtml

Orphanet:

http://www.orpha.net/consor/cgi-bin/index.php

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