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# A rapid immunohistochemical test to distinguish congenital myotonic dystrophy from X-linked myotubular myopathy

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**Abstract**

Severe forms of myotubular myopathy (MTM) and congenital myotonic dystrophy type 1 (CDM), both present as floppy infants with hypotonia, respiratory failure and bulbar insufficiency. Muscle biopsy is often performed as part of the diagnostic process, but these two disorders share very similar histopathological features. It is well documented that CDM muscle has nuclear foci that contain muscleblind-like 1 (MBNL1) protein. In muscle biopsies from eight neonates showing central nuclei, MBNL1 immunolocalisation identified discrete, intensely stained foci in three cases that were subsequently confirmed as CDM by DNA analysis. In the five remaining non-CDM patients and two controls, MBNL1 staining was heterogeneous in nuclei, not as foci. MBNL1 staining patterns in CDM were easily distinguishable from MTM. We suggest that in cases of hypotonia with suspected CDM or MTM, when biopsy has been taken, sections should additionally be stained for MBNL1 to provide a rapid indication of a CDM diagnosis.


*Keywords:* Congenital myotonic dystrophy; Myotubular myopathy; Hypotonia; Floppy infant; Muscleblind-like protein; MBNL1

## 1. Introduction

There are many possible causes of neonatal hypotonia and accurate diagnosis requires careful systematic clinical examination and a broad range of investigations [1]. A review of three clinical series of hypotonic infants reported that congenital myopathies accounted for 5% and congenital myotonic dystrophy (CDM) for 4% of all cases [1].

Pathological mutations of the myotubularin 1 (*MTM1*) gene on chromosome Xq27.3-q28 give rise to the X-linked recessive form of myotubular myopathy (MTM) [2,3]. A large number of different mutations in *MTM1* have been identified which may be missense, nonsense, insertions, deletions or splice site mutations. Male infants with the X-linked form are usually severely affected and present with respiratory failure, bulbar weakness, joint contractures and muscle weakness. Female carriers show variable clinical severity which may range from minimal signs to severe neonatal hypotonia due to skewed X-inactivation [3]. Autosomal forms of myotubular myopathy have also been described which may be associated with mutations in *DNM2*, *BIN1*, or *RYR1* and possibly also *MTMR14* [4–7]. Autosomal myotubular myopathy often presents later and has a milder phenotype compared with the X-linked form, but there are examples of severe and early onset cases [8].

The severe congenital form of myotonic dystrophy (CDM) occurs only in myotonic dystrophy type 1 (DM1), caused by dominant trinucleotide (CTG) repeats in the 3′-untranslated region of the *DMPK* gene on chromosome 19q13.3 [9]. Resultant mutant RNA with CUG repeats has a toxic gain of function which leads to misregulated alternative splicing and other events such as altering

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transcription and translation [10–12]. The severity of DM1 tends to increase from one generation to the next, a phenomenon know as "anticipation", which is due to CTG expansions in the germline, with the largest expansions occurring during maternal transmission. The size of repeat expansion in DM1 is correlated with clinical severity. Newborn babies with congenital DM1 are often born prematurely and are hypotonic and weak with joint contractures and have difficulty with sucking, swallowing and breathing. A positive family history of myotonia, particularly in the mother, is a helpful indicator in the diagnosis of CDM, but before birth the family history will often not have been established. A confident diagnosis of CDM may be obtained by careful maternal assessment and subsequent genetic testing, the results of which may not be available for several weeks A second form of myotonic dystrophy, which does not have a congenital presentation, is myotonic dystrophy type 2 (DM2), caused by expansion of a tetranucleotide (CCTG) repeat in the *CNBP* gene [13].

\Therefore, MTM and CDM regularly present with the same symptoms and often the two conditions cannot be easily distinguished clinically. In addition, muscle histopathology of these two conditions is very similar, the predominant feature being an abundance of large central internal nuclei. Thus molecular genetic analysis is often essential to make the diagnosis, which may take several weeks to obtain.

In DM1, much of the RNA with the large CUG repeats is spliced and polyadenylated normally and accumulates in the nuclei as discrete foci [14,15], often at the periphery of nuclear splicing speckles [16]. Muscleblind-like 1 (MBNL1) protein binds with high affinity to the expanded repeats and has been reported to concentrate at the ribonuclear foci in DM1 skeletal muscle [17–21], DM1 cortical and subcortical neurons [22], DM1 cardiac muscle [23], DM1 buccal cells [24] and in cultured DM1 fibroblasts and myoblasts [16,21,25–27], thereby leading to a reduction in the local nucleoplasmic levels of MBNL1. Muscleblind-like 1 (MBNL1) protein is a regulator of alternative splicing. Observations suggest that when MBNL1 binds upstream of an exon, it acts as a splicing suppressor and when it binds downstream of an exon, it may act as a splicing enhancer [11,28].

The aim of the present study was to perform immunohistochemistry for MBNL1 on muscle biopsy sections from newborn hypotonic infants to determine whether sequestration of MBNL1 to ribonuclear foci could distinguish myotonic dystrophy from other conditions with abundant central nuclei and to confirm the results with in situ hybridisation.

## 2. Materials and methods

### 2.1. Biopsy material

Biopsies of quadriceps muscle were obtained for diagnosis after informed parental consent from 7 hypotonic newborn infants with central nuclei, aged 5–86 days and one foetus of 21 weeks gestation. These were compared with biopsies from two non-hypotonic control patients that were sampled as part of an ethically approved study. Patient details are shown in Table 1.

Biopsy material was frozen in isopentane cooled in liquid nitrogen, according to standard procedures [29] and 5 μm serial cryostat sections cut onto glass slides. Procedures applied included haematoxylin and eosin, Gomori trichrome stains, stains for oxidative enzymes and a panel of immunohistochemical studies, including antibodies to myosin isoforms and various sarcolemmal proteins, according to standard procedures [29].

### 2.2. MBNL1 immunolabelling

Sections were fixed with 1% formalin in PBS for 10 min, washed with PBS, blocked with 1% glycine in PBS for 10 min and again washed with PBS. Monoclonal antibody MB1a against MBNL1 [16] was diluted 1:3 in PBS and incubated on sections for 1 h, followed by washing with PBS. Monoclonal antibody MB1a, available from the MDA Monoclonal Antibody Resource (www.glennmorris.org.uk/mabs.htm) recognises an epitope containing amino acid sequence "VSPSL" which is present in the linker region, between the paired zinc finger motifs of MBNL1. Sections were then incubated for 1 h with 5 μg/ml goat anti-mouse ALEXA 488 (Molecular Probes, Eugene, Oregon, USA) in PBS containing 1% horse serum, 1% foetal bovine serum and 0.1% BSA. DAPI (diamidino phenylindole) was added for the final 10 min of incubation to counterstain nuclei before washing and mounting in Hydromount (Merck).

### 2.3. Fluorescence in situ hybridisation (FISH)

For combined immunohistochemistry and FISH [21], primary and secondary antibody incubations were completed, followed by treatment with 4% paraformaldehyde, 5 mM $MgCl_2$ in PBS for 10 min and washing with 2× SSC (300 mM sodium chloride, 30 mM sodium citrate pH 7). Sections were then treated with 40% deionised formamide in 2× SSC for 5 min, which was removed prior to addition of the in situ hybridisation mix (10% dextran sulphate, 40% formamide in 2× SSC containing: 0.2% bovine serum albumin, 0.1 mg/ml herring sperm DNA, 0.1 mg/ml baker's yeast transfer RNA (Sigma), 4 mM ribonucleoside vanadyl complexes (Sigma) and a DM1 probe: 200 nM Cy3-labelled (CAG)10 oligonucleotide (Qiagen Operon, Cologne, Germany). Slides containing in situ hybridisation mix were incubated in a humidified chamber for 16 h, followed by thorough washing in 2× SSC, counterstaining with DAPI for 10 min and mounting in Hydromount.

### 2.4. Microscopy

Sections were viewed and assessed independently and without prior knowledge of the diagnosis by two observers