Independent Regulation of the Myotonic Dystrophy 1 Locus Genes Postnatally and during Adult Skeletal Muscle Regeneration*

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Myotonic dystrophy is caused by a CTGₙ expansion in the 3'-untranslated region of a serine/threonine protein kinase gene (DMPK), which is flanked by two other genes, DMWD and SIX5. One hypothesis to explain the wide-ranging effects of this expansion is that, as the mutation expands, it alters the expression of one or more of these genes. The effects may vary in different tissues and developmental stages, but it has been difficult to develop these hypotheses as the normal postnatal developmental expression patterns of these genes have not been adequately investigated. We have developed accurate transcript quantification based on fluorescent real-time reverse transcription-polymerase chain reaction (TaqMan) to develop gene expression profiles during postnatal development in C57Bl/10 mice. Our results show extensive independent postnatal regulation of the myotonic dystrophy-locus genes in selected tissues and demonstrate which are the most highly expressed of the genes in each tissue. All three genes at the locus are expressed in the adult lens, questioning a previous model of cataractogenesis mediated solely by effects on SIX5 expression. Additionally, using an in vivo model, we have shown that Dmpk levels decrease during the early stages of muscle regeneration. Our data provide a framework for investigation of tissue-specific pathological mechanisms in this disorder.

Myotonic dystrophy (DM1) is an autosomal dominant neuromuscular disorder with very variable symptom presentation. Anticipation (a decrease in the age of onset with transmission) is often seen in DM1 and is usually accompanied by increased symptom severity (1). The most severe manifestation of the disease is the congenital form, in which newborns present with generalized skeletal muscle hypotonia. In marked contrast, the most minimally affected patients may simply develop late-onset cataracts (clinical presentation reviewed in Ref. 1). The mutation underlying DM1 is a CTGₙ expansion on chromosome 19 (2–7). In the normal population the repeat is between 5–37 copies, but in the DM1 population this may reach several kilobases in peripheral blood leukocytes (8). The repeat is mitotically and meiotically unstable, and there is a broad but not absolute correlation between the expansion size and symptom severity, providing a molecular basis for anticipation (9–11).

There is no consensus on how the mutation causes the complex and varied clinical manifestation in DM1. A number of hypotheses have been advanced, including the titration and sequestration of specific RNA-binding proteins (12–14) or the formation of abnormal chromatin structures (15–17). Most attention has focused on the effects of the expansion on nearby genes. The CTG repeat lies in the 3'-untranslated region of a serine/threonine protein kinase (DMPK) (3). A homeodomain gene of the SIX family (SIX5, formerly DMAHP) (18) lies less than 1 kilobase from the 3’ end of DMPK, and a WD-repeat gene (DMWD, formerly 59) (19) terminates less than 1 kilobase from the 5’ end of DMPK. It has been hypothesized that the expansion may influence the expression of these DM1-locus genes and that this effect could vary in different tissues and at different developmental stages. Allele-specific decreases in the expression of the DM1 locus genes in tissues derived from DM1 patients have been reported (20–23), although a recent study reported that overall expression of the transcripts fell within the control population range (24).

If gene dosage at the DM1-locus contributes to the development of the phenotype, its effects may vary in different tissues, depending on the basal expression of each gene. Existing data on expression of the DM1-locus genes is mainly based on relatively insensitive techniques e.g. Northern blotting, which is insufficient to detect SIX5 expression in most tissues or non-quantitative RT-PCR. Additionally, there have been no truly systematic studies addressing the alterations in expression occurring postnatally, which may be significant given the very different presentations in the congenital and adult forms of the disorder and the late-onset of some of the symptoms.

To address this we have examined the expression of the DM1 locus genes in a range of tissues from neonatal and mature mice to generate a spatial and temporal map of expression from this highly conserved locus. To obtain genuinely quantitative data for a large number of samples, it is essential to use a technique that is sensitive, accurate, and has high throughput. The TaqMan system (Perkin-Elmer), which measures fluorescence in real time, fulfils all these requirements and was used throughout.

Additionally, it has been reported that in tissue culture systems high levels of DMPK or its 3'-untranslated region inhibit differentiation of myoblasts to mature myotubes (25, 26). It was unclear how, if at all, this related to muscle physiology in vivo. By using an in vivo experimental model of skeletal muscle...
regeneration in mice, we have extended the physiological sig-
nificance of these findings.

**EXPERIMENTAL PROCEDURES**

*Mouse Tissue Samples*—Wild type C57Bl/10 mice were sacrificed by cervical dislocation, and samples were removed, snap-frozen in liquid nitrogen, and stored at −70 °C until processed. The tissue samples were collected from neonatal and adult (10 weeks of age) mice. In some cases, samples from juvenile mice (3 weeks of age) were also analyzed. 3 males and 3 females were dissected at each time point.

*In Vivo Regeneration Samples*—Skeletal muscle regeneration was induced in the tibialis anterior muscle of 4-week-old male C57Bl/10 mice. 50 μl of 1.2% BaCl2 in Hanks' balanced salt solution were injected into the right tibialis anterior; 50 μl of Hanks' balanced salt solution alone were injected into the left tibialis anterior as a control. The mice (3 per time point) were sacrificed at 3, 10, and 25 days post-injection, and the tibialis anterior muscles were removed. One-third of each muscle was snap-frozen in liquid nitrogen for TaqMan, and the remaining two-thirds were mounted in Bright Cryo-M-Bed (Bright Instrument Co., Ltd.) and frozen in isopentane cooled to −165 °C in liquid nitrogen.

*mRNA Extraction and cDNA Synthesis*—Cytoplasmic poly(A) mRNA was isolated from 10–40 μg of tissue using the Invitrogen Micro Fast Track mRNA isolation kit. 70% of the isolated mRNA was used as a template for the production of first-strand cDNA using random primers (cDNA Cycle Kit, Invitrogen). The cDNA was resuspended in 50 μl of nuclease-free sterile distilled water. 14 μl of each cDNA was mixed with 56 μl of sterile distilled water, and this diluted preparation was used as the template for each TaqMan assay for the 4 genes. The remaining 30% of the mRNA was used as the template for a no-reverse transcrip
tase control.

**TaqMan Assays**—The TaqMan system is based on Taq polymerase 5'-3' nuclease activity (27), which cleaves a dually labeled non-exten
dible TaqMan probe designed to hybridize to a sequence between the forward and the reverse primer for every particular amplicon. The probe has a quencher dye on its 3' end and a reporter dye at the 5'. Fluorescence emission from the reporter is quenched by the quencher dye found on the reporter to separate the two dyes and enable detection of the reporter dye fluo
rescence (28, 29). Primers and probes for the four different amplicons were designed using the Primer Express (Perkin-Elmer) and Oligo 5.0 software (National Biosciences). The amplicons were designed to have one primer covering an exon-exon boundary. Primer sequences (Integrated, Ulm, Germany; high performance liquid chromatography-puri
fied), 5'-caccagacgacctctcc-3' and 5'-gtgataagcgtctgat-3'; *Tbp* (exons 3/4), 5'-ggaaataattctggctcatagctactg-3' and 5'-gagaagatctgcagcagctg-3'; *Dmd* (exons 2/3), 5'-gtgataagcgtctgat-3'; *Six5* (exons 3/4), 5'-agttc-3' and 5'-0.3 units. For Ct values
for each dATP, dCTP, and dGTP, 400 nM dUTP, 100 nM TaqMan probe, and 6-carboxyfluorescein (FAM) and 6-carboxytetramethylrhodamine (TAMRA), respectively. The Taq
Molecular analysis (data not shown), demonstrating that the assays were consistent with the expected number of copies in the unknown samples by comparison with standards.

To obtain absolute quantification, as desired in this study, it is essential to incorporate standard curves for every assay. Standard curves for each amplicon were plotted from 5 different concentrations of standards run in triplicate and were considered acceptable if the correlation coefficient was >0.98 (correlation coefficient and S.D. among the triplicates calculated using Sequence Detector Software). For Ct values <30, triplicates were not allowed to differ by more than 0.3 units. For Ct values >30 the variation was not allowed to exceed 0.5 Ct. For any samples exceeding these limits, the entire TaqMan assay was repeated at least 3 times.

The expression data for all samples were normalized to the levels for an established (30) housekeeping gene *Tbp* (TATA-binding protein). Transcript levels of this gene remain constant, with alterations in *Tbp* protein levels in certain adult tissues mediated by translational/post-
translational mechanisms (31). The only known exception to this is adult testis in which there are higher levels of *Tbp* mRNA (32), neces
sitating a conversion factor of 18. The copy numbers for the DM1-locus genes were normalized to the copy numbers obtained for *Tbp* by divid
ing the mean of the triplicates for the test genes with the mean of the triplicates of *Tbp* for each individual sample.

**Standard Curves**—The four individual amplicons were amplified from a liver cDNA preparation using standard PCR methodologies, purified, and individually cloned into the PCR TOPO 2.1 cloning vector (Invitrogen, Netherlands). The cloned fragments were sequenced to confirm that the insert was the correct sequence and present in only a single copy. Purified plasmid DNA was prepared from 300-mL cultures using stages 1 to 4 of the Qiagen Plasmid Midi kit, filtered, precipitated in absolute ethanol, washed in 70% ethanol, resuspended in 1 ml of 10 mM Tris, 1 mM EDTA, pH 8 buffer, and further purified by standard CsCl centrifugation and dialysis. Plasmid concentrations were deter
mined by ultraviolet spectrophotometry, and purity was confirmed by agarose gel electrophoresis. Purified clones were diluted and stored in single-use aliquots at −20 °C, and the same diluted preparations were used throughout.

**Histology and Fiber Counts of Regenerating Muscle Samples**—Muscles for histological analysis were mounted transversely, and 8-μm thick cryostat sections were cut and stained with hematoxylin and eosi
cin. The number of fibers containing peripheral or central nuclei was counted by light microscopy.

**Statistics**—As the sample groups were small, comparisons were performed using the non-parametric Mann-Whitney test. Data are stated as showing a significant difference if *p* < 0.05.

**RESULTS**

Following initial optimization of the PCR protocols, all primer pairs successfully amplified single products from cDNA, of the expected sequence. No products were detected with genomic DNA template or when excess no-RT controls were analyzed (data not shown), demonstrating that the assays were specific for the transcribed products. Analyses of different di
lutions of selected cDNA preparations on different days and using freshly made reaction mixes showed that the variation between assays for individual samples did not exceed 5%. The standard curves demonstrated linearity of the assays with no change in the amplification efficiency over the tested range of 12 up to 1,200,000 copies of template.

**Postnatal Expression of the DM1-locus Genes**—Fig. 1 summarizes the expression of the DM1-locus genes in both neonatal and adult tissue samples. *Dmpk* is the gene whose expression changes most markedly during postnatal development, in
creasing in the male distal and proximal skeletal muscles, the heart, and the cerebral cortex and cerebellum. In females there is also an increase in *Dmpk* expression in the distal skeletal muscles, the heart, and the small intestine but no significant postnatal changes in the other tissues (see “Discussion” for further information on the small intestine data for the male mice). Postnatal up-regulation of *Dmd* was also observed.
FIG. 1. Neonatal and adult expression of the DM1-locus genes in C57Bl/10 mouse tissues. Tissue samples are listed on the vertical axis. D, distal skeletal muscles; P, proximal skeletal muscles; H, heart; Co, brain cortex; Ce, brain cerebellum; Li, liver; G, gonads; Si, small intestine; Le, lens. Mean levels of expression of the DM-locus genes, normalized to Tbp, are displayed logarithmically on the horizontal axis. Panels A–C, males; panels D–F, females. Panels A and D, Dmpk; panels B and E, Dmwd; panels C and F, Six5. Empty bars, neonatal; filled bars, adult. p < 0.05 is marked with an asterisk. For clarity, only positive error bars (S.D.) are shown. For all categories, n = 3 mice, except for adult male small intestine, where n = 2 (see “Discussion”).

Dmwd was up-regulated in the male adult distal skeletal muscles, cerebral cortex, and testis, but in contrast, Dmwd levels decreased postnatally in the female cerebral cortex. A similar postnatal decrease was seen in the female small intestine. Six5 expression was down-regulated in adult male proximal skeletal muscles and testis compared with the neonates, but expression increased postnatally in the cerebral cortex. In female mice the only significant change detected for Six5 was a postnatal increase in expression in the small intestine.

Patterns of Expression of the DM1-locus Genes—In Fig. 2 the graphs demonstrate the patterns of DM1-locus gene expression in different mouse tissues. In certain tissues Dmpk is strikingly much more highly expressed than the other genes. In the distal and proximal skeletal muscles, the small intestine, and the adult heart the levels of Dmpk transcripts far exceed those of Dmwd and Six5 by as much as 2 orders of magnitude. The Dmpk expression in these tissues is also much higher than the levels of Dmpk in the other tissues sampled, e.g. Dmpk is approximately 50-fold more highly expressed in the adult female heart than in the ovaries. It is also notable that in some tissues Dmpk is not the most highly expressed of the genes. In neonatal cortex and cerebellum, Dmwd is more highly expressed than the other two genes, although this is less apparent in the adult samples, except for the female cortex. Dmwd is also the most highly expressed of the DM1-locus genes in the adult testis. There is no tissue in which Six5 is the most highly expressed of the three genes, and in the majority of samples it is expressed at a very low level, particularly in the neonatal brain and the adult testis. Due to technical constraints, we did not dissect out the lens from neonatal mice and, therefore, do not have development data for this tissue. However, it is apparent that all three DM1-locus genes are expressed in the adult lens.

DM1-locus Gene Expression during in Vivo Skeletal Muscle Regeneration—Fig. 3 shows the results obtained after in vivo regeneration of the tibialis anterior muscle. Regenerated fibers are characterized by central nuclei. 3 days post-injection the BaCl2-treated muscles consisted mainly of newly regenerated small diameter, twitch-type fibers interspersed with connective tissue and some peripherally nucleated fibers that had not undergone regeneration. Counts of central nucleation were not recorded at 3 days because of the disparity in fiber size and presence of connective tissue compared with the later time points. 10 days post-injection treated muscles contained centrally nucleated fibers (35–45%) of increased diameter, with minimal amounts of surrounding connective tissue compared with the earlier time point. A similar pattern was seen at 25 days post-injection (16–27% of fibers centrally nucleated). Control contralateral muscles at all 3 time points consisted of close-packed muscle fibers of uniform diameter with virtually no regeneration (data not shown). At 3 days post-treatment Dmpk levels in the regenerating muscles were significantly lower than in the controls. This was the only significant difference seen in gene expression during in vivo regeneration.

DISCUSSION

This report is the most extensive study addressing the issue of postnatal expression patterns and developmental regulation of the DM1 locus genes and the only one to employ genuinely quantitative methodologies. The majority of previously published studies have analyzed expression of only one or, at most, two of the DM1-locus genes, making it very difficult to compare relative levels of gene expression, e.g. to determine if in a particular tissue Dmpk is more highly expressed than Dmwd or how expression of a gene varies between tissues. In contrast, in this report we have used the highly quantitative and reproducible technique of fluorescent real-time RT-PCR, the most sensitive method of quantification available for high throughput studies, which is rapidly becoming the technique of choice in expression studies. Because of its extended linear detection range, the same technique can be used to assess accurately transcript levels from genes that are expressed at widely varying levels, an advantage not enjoyed by the methods previously applied to the DM1-locus genes. Additionally, by including a standard curve for the relevant amplicon on every plate and analyzing all genes for a tissue on a single diluted cDNA preparation, we have abrogated the effects of any inter-test variation in amplification efficiency.

It is clear that there is a complex set of postnatal changes in expression of the DM1-locus genes, and the genes are clearly independently regulated during postnatal development. The most striking changes are seen for Dmpk. Expression of Dmpk increases postnatally in skeletal muscles (with the possible exception of distal muscles in females), the heart, and the small intestine in females. It appears from Fig. 1 that similar increases occur in the male small intestine, but we cannot perform statistical analyses as n = 2 for the adult small intestine (despite repeated extractions and assays, the Tbp data for this sample failed to match our selection criteria). It is noteworthy that these increased levels of Dmpk expression occur in tissues rich in either striated or smooth muscle. The workload of the muscle cells in all these tissues increases considerably during
postnatal development, and it may be inferred that increased levels of Dmpk are required under these conditions. Whether this increase is mediated through mechanotransductive processes or is a consequence of changing hormonal influences remains to be established. Preliminary data from juvenile mice (3 weeks old) suggested that in distal skeletal muscles and hearts much of this up-regulation was established within the first few weeks of birth (data not shown). The increased expression of Dmpk in these samples does not represent merely a general pattern in which expression of all non-housekeeping genes is up-regulated, as it is noticeable that expression of Dmwd in the small intestine falls dramatically postnatally.

Expression of the DM1-locus genes in the cerebral cortex also seems to exhibit significant levels of postnatal regulation. In all male mice there is significant up-regulation of each gene in the adult cortex compared with neonates, but the same effect is not observed for female mice, where levels of Dmwd drop significantly postnatally. It is not known if these gender-specific differences represent a physiologically relevant phenomenon, perhaps related to hormonal influences.

The postnatal expression of the DM1-locus genes does not alter significantly in the ovaries, in contrast to the testis. Levels of Dmwd are significantly increased in the adult testis compared with the neonates, but the opposite trend is observed for Six5. Dmpk levels in the testis do not change postnatally, again reinforcing the concept that there is no obligatory co-ordinate regulation of the genes at this locus.

As our technique is standardized, we can calculate definitively the absolute expression patterns of the DM1-locus genes within and between samples. In the skeletal muscles, heart, and small intestine, expression of Dmpk far outstrips that of Dmwd or Six5, in some cases by more than 2 orders of magnitude. In the other tissues analyzed, there are fewer major differences between the expression of the genes, with the exception of the cerebral cortex and adult testis, in which Dmwd is the most highly expressed of the DM1-locus genes. It has

**FIG. 2.** Relative expression of the DM1-locus genes in C57Bl/10 mouse tissues. Tissue samples are shown on the horizontal axis. D, distal skeletal muscles; P, proximal skeletal muscles; H, heart; Co, brain cortex; Ct, brain cerebellum; Li, liver; G, gonads; Si, small intestine; Le, lens. Mean levels of expression of the DM-locus genes, normalized to Tbp, are displayed logarithmically on the vertical axis. A, neonatal males; B, neonatal females; C, adult males; D, adult females. Solid black bars, Dmpk; empty bars, Dmwd; hatched bars, Six5. For clarity, only positive error bars (S.D.) are shown. For all categories, n = 3 mice, except for adult male small intestine, where n = 2 (see “Discussion”).

**FIG. 3.** Expression of the DM1-locus genes during in vivo muscle regeneration. Mean levels of expression of the DM-locus genes, normalized to Tbp, are displayed logarithmically on the vertical axis for days 3, 10, and 25 post-treatment (A–C). On the horizontal axis, the genes assayed are shown and the experimental conditions (+ denotes BaCl2, − denotes control). For clarity, only positive error bars (S.D.) are shown, and n = 3, p < 0.05 is marked with an asterisk. Hematoxylin and eosin sections of the BaCl2-treated muscles are shown at the same time points. R, regenerating areas; NR, non-regenerating. Scale bar, 50 μm.
been suggested for some time that \textit{Dmpkd} has its highest expression levels in these tissues (19), but ours is the first report showing that these levels outstrip those of the other DM1-locus genes. In all adult tissues it was noticeable that \textit{Six5} was never the most highly expressed gene, and in some tissues its expression was markedly reduced compared with \textit{Dmpkd} and \textit{Dmpwd}, although we cannot exclude the possibility that there may be small subsets of cells overexpressing a particular gene in a heterogeneous tissue sample. Cell-specific expression can only be determined by \textit{in situ} hybridization or immuno-histochemistry if suitable antibodies become available, but neither of these techniques is genuinely quantitative. The other alternative, of micro-dissection of samples prior to the TaqMan analyses, is not technically feasible for this work.

Our data also indicate that, unlike \textit{Dmpkd} and \textit{Six5}, expression of \textit{Dmpwk} is influenced by the regenerative status of the muscle. \textit{Dmpkd} is down-regulated during skeletal muscle regeneration, and taken together with the previously published cell culture work (25, 26), this suggests that very elevated levels of this transcript may be incompatible with the fusion of myoblasts to the terminally differentiated myotube state. This may introduce additional difficulties in analyzing \textit{DMPK} expression data from patients with DM1, as it will be important to determine if apparent changes in levels of \textit{DMPK} in skeletal muscles between DM1/non-DM1 patients are actually a primary molecular effect or a secondary consequence of increased regeneration in a particular sample.

What are the implications of the data published here for an understanding of the pathology observed in DM1? Probably the most clear-cut conclusions can be drawn from the data for the adult lens. Winchester \textit{et al.} (33) analyzed the expression of \textit{SIX5} and \textit{DMPK} in the human lens using \textit{in situ} hybridization and RT-PCR. They were unable to detect transcripts for \textit{DMPK} and concluded that \textit{SIX5} was thus a much stronger candidate for involvement in the formation of the characteristic cataracts that are such a common feature in this disease. In contrast, transcripts for all three DM1-locus genes were detected in our adult mouse lens samples. This is unlikely to reflect contamination by other adherent tissues. Samples were collected by a veterinarian with considerable experience of mouse anatomy, and every effort was taken to minimize tissue contamination compatible with minimizing the time before samples were frozen. It is also unlikely that our data represent a simple cross-species difference. In our hands transcripts for all three genes can be readily detected by RT-PCR in freshly isolated human primary lens epithelial cells,\(^2\) casting considerable doubt on any model of cataract formation solely based around \textit{SIX5}.

DM1 has a very complex multisystemic phenotype and may be more correctly described as a single-locus than a single-gene disorder, \textit{i.e.} functional haploinsufficiency for one or more of the genes may vary between tissues and ages, depending on the physiological threshold for each gene product. However, attempts to identify molecular pathological mechanisms in DM1 based on models of gene dosage have been severely hampered by the lack of background data available on the normal expression patterns of the DM1-locus genes. This paper attempts to address this by providing the first genuinely quantitative data that allows cross-comparisons between the different genes. Although this work was carried out in a model organism rather than in human material, it provides a useful starting point for generating a developmental map of expression.

During mapping efforts to identify human disease genes, it is quite common to be faced with a number of candidate genes from a defined genetic interval. Under these circumstances, a common strategy is to investigate which of the candidates shows the closest correlation between the expression of the gene and the expression of the phenotype. The same rationale can be applied to the DM1 locus, whereby we have three candidate genes for which we need to determine if one of them shows a close correlation between phenotype and expression. Intriguingly, such a correlation appears to exist between the tissues most commonly involved in adults with DM1 and \textit{Dmpwd}. Its levels are highest in lens, testis, and heart and lowest in the tissues rarely implicated, \textit{e.g.} ovaries and liver (although DM1 patients are often insulin-resistant, this is a purely skeletal muscle phenomenon, as the hepatic response to insulin is normal (34)). The high expression in the lens may be significant, as this tissue acts as the most sensitive indicator of DM1 pathology in minimal expansion patients. Such a correlation is less apparent for the other genes, \textit{e.g.} \textit{Dmpkd} levels are lowest in the adult gonads, cerebral cortex, cerebellum, and lens, all of which, with the exception of the ovaries, are commonly implicated in DM1. \textit{Six5} levels in adults are higher in the liver than the cerebral cortex or testis, inconsistent with the DM1 phenotype. \textit{Dmpkd} levels are also very low in the small intestine, but the pathological data on the small intestine is difficult to interpret. Gastro-intestinal disturbances are commonly reported in DM1 with abnormalities in the large bowel (35) and the duodenum (36) (not analyzed in our study) clearly demonstrated, but more research is required to determine the extent to which the rest of the small intestine is involved. Our data suggest this issue of the precise patterns of tissue involvement in the phenotype may be important. The apparent correlation of symptom presentation with \textit{Dmpkd} expression is intriguing, but unfortunately, very few molecular studies have analyzed the expression of \textit{Dmpkd} in patient material. Although our own work suggests that the cytoplasmic levels of \textit{DMWD} transcripts in skeletal muscle may fall within those found in the control population (24), an allele-specific decrease in \textit{DMWD} transcripts from the expanded allele has been reported (22). The data here suggest that this gene may warrant closer examination than it has previously been awarded to establish conclusively if in a range of DM1 patient tissues, including individual muscle fibers, there is a genuine decrease in \textit{DMWD} transcripts. As there is currently no data on the activities and intracellular functions of DMWD protein, it is difficult to speculate at this stage on potential pathological pathways that might result if there is haploinsufficiency of this gene product in patient tissues.

In summary, our data have shown that in both muscle regeneration and postnatal expression there is independent regulation of the three DM1-locus genes. This has implications for developing molecular models of DM1, including differentiating between mechanisms for congenital and adult forms of the disorder. Although the very high level of expression of \textit{Dmpkd} suggests it has an important role in adult skeletal muscle function, it is down-regulated during muscle regeneration. Additionally this research demonstrates that \textit{SIX5} is not the sole candidate for cataractogenesis and that \textit{DMWD} may be a stronger candidate for involvement in many aspects of the adult phenotype than has generally been recognized.

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\textbf{REFERENCES}

1. Harper, P. S. (1989) \textit{Myotonic Dystrophy}, 2nd Ed., W. B. Saunders Co. Ltd., London

2. Aslanidou, C., Jansen, G., Amemiya, C., Shutler, G., Mahadevan, M., Tsilfidis, C., Chen, C., Alleman, J., Wormskamp, N. G., Voojis, M., Buxton, J., Johnson, K., Smeets, H. J. M., Lennon, G. G., Carrano, A. V., Korneluk, R. G., Wieringa, B., and De Jong, P. (1992) \textit{Nature} 355, 548–551

\(^2\) N. Carey, manuscript in preparation.
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