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ORIGINAL PAPER

# Overexpression of Myostatin2 in zebrafish reduces the expression of dystrophin associated protein complex (DAPC) which leads to muscle dystrophy

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**Abstract** Myostatin, a member of the TGF-$\beta$ superfamily, is a potent negative regulator of skeletal muscle and growth. Previously, we reported Mstn1 from zebrafish and studied its influence on muscle development. In this study, we identified another form of Myostatin protein which is referred to as Mstn2. The size of Mstn2 cDNA is 1342 bp with 109 and 132 bp of 5′ and 3′-untranslated regions (UTRs), respectively. The coding region is 1101 bp encoding 367 amino acids. The identity between zebrafish Mstn1 and 2 is 66%. The phylogenetic tree revealed that the Mstn2 is an ancestral form of Mstn1. To study the functional aspects, we overexpressed *mstn2* and noticed that embryos became less active and the juveniles with bent and curved phenotypes when compared to the control. The RT-PCR and in situ hybridization showed concurrent reduction of dystrophin associated protein complex (DAPC). In cryosection and in situ hybridization, we observed the disintegration of somites, lack of transverse myoseptum and loss of muscle integrity due to the failure of muscle attachment in *mstn2* overexpressed embryos. Immunohistochemistry and western blot showed that there was a reduction of dystrophin, dystroglycan and sarcoglycan at translational level in overexpressed embryos. Taken together, these results indicate the suitability of zebrafish as an excellent animal model and our data provide the first in vivo evidence of muscle attachment failure by the overexpression of *mstn2* and it leads to muscle loss which results in muscle dystrophy that may contribute to Duchenne syndrome and other muscle related diseases.

**Keywords** Myostatin · Muscle attachment · Muscular dystrophy · DAPC · Muscle development

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## Introduction

A number of genetic factors, hormones and nutritional factors are important for skeletal muscle mass [1]. However, their precise role in the integrated, in vivo regulation of skeletal muscle homeostasis and muscle wasting associated with chronic illness and aging remains poorly understood. Considerable interest has been focused on the role of *mstn* [2, 3], a novel regulator of muscle mass generally expressed in skeletal muscle. The *mstn* (growth and differentiation factor-8) belongs to transforming growth factor-$\beta$ super family, that plays as a negative growth regulator for skeletal muscle, such that mutations in the *mstn* gene result in a hypermuscular phenotype in mice

and cattle [4, 5]. However, influence of Mstn on muscle phenotypes is still unclear. Homozygous disruption and knockdown of *mstn* gene in mice [2] and fish [6] result in a significant increase of skeletal muscle mass and a reduction of body fat. The *mstn* is expressed in cells of the skeletal muscle lineage throughout embryogenesis beginning in the myotome compartment of developing somites [7]. Overexpression of *mstn* leads to muscle wasting in adult mice [8, 9]. Besides, changes in muscle mass due to disease or misuse are often reflected by changes in *mstn* expression [10, 11]. Skeletal muscle mass is lost in genetic disorders such as muscular dystrophy, muscle wasting and aging [12].

Mstn shares several structural similarities to other TGF-$\beta$ family members. It contains a hydrophobic N-terminus that acts as a secretary signal and a conserved RSRR domain that is important for proteolytic processing [5, 3, 13]. Cleavage of protein gives rise to an N-terminal latency associated peptide and C-terminal mature signaling peptide. The mature signaling peptide binds to the receptor, suggested to be activin type II receptor, to elicit its biological function [14]. Indeed, recombinant Mstn based on the mature signaling portion has been shown to be biologically active in repressing the proliferation of cultured myoblast cells [15].

In many aspects *mstn* in the fish are quite different from that of higher vertebrates. Studies in mice and cattle revealed that *mstn* mRNA is specifically expressed in developing somites and skeletal muscles [5, 4]. Duplication of *mstn* gene in Atlantic salmon [16] and differential expression of *mstn* gene in both muscular and non-muscular tissues in Asian sea bass [17] have been observed. Recent studies, however, showed that in addition to muscle cells, *mstn* mRNA also expressed in several other tissues, such as cardiomyocytes, mammary glands and, at a lower level, in adipose tissue [18]. In fish, *mstn* mRNA was found to be expressed in muscles, eyes, gill filaments, spleen, ovaries, gut, and brain and, to a lesser extent, in testes [19, 6]. Roberts and Goetz [20] reported that *mstn* mRNA primarily expressed in red muscles in brook trout, king mackerel, and yellow perch, but expression in the little tunny is in the white muscles. Two distinct forms of *mstn* genes have been demonstrated in Atlantic salmon [21], rainbow trout [22], and seabream [23]. Unlike mammals, in fish *mstn* is expressed in several tissues in addition to the muscle.

To understand the role of *mstn* in fish skeletal muscle development and growth, here we have cloned and studied the relationship between the *mstn1* and *mstn2* of zebrafish. To investigate more about the functional aspects, we overexpressed the *mstn2* and observed embryos with bent morphology and lower activity [24], an interesting homologue of mammalian muscle dystrophy.

## Materials and methods

### Fish culture and maintenance

Routine fish care and maintenance were performed as described [25]. Zebrafish embryos were collected and staged in hours post fertilization (hpf) at standard temperature (28.5°C).

### Cloning and sequence analysis

We used human GDF-8 (*mstn*) mRNA sequence (Accession number, NM_005259) as virtual probe to BLAST against zebrafish genomic database (Sanger Center, Zebrafish assembly v2) and found zebrafish genomic clone (zfish41364-181h04.q1c) which was highly homologous to GDF8, containing the recognizable domains shared by TGF-$\beta$ family, but different from the previously reported zebrafish *mstn* (Accession number, NM_131019). According to this genomic information, we designed gene specific primers and performed the whole tissue of 5′ RACE/3′ RACE to amplify the *mstn2* mRNA sequence. Total RNA was extracted from adult zebrafish using TRIZOL reagent (Invitrogen). First strand cDNA was synthesized using GeneRacer kit (Invitrogen) to obtain a full length cDNA and subjected to PCR. The reverse transcribed sample (2 µl) was used in a 50 µl PCR mixture. The primers for first PCR were GeneRacer 5′ Primer and *zfmstn2*-GSP1 (5′-TCA AGA GCA ACC GCA AAG GTC TAC CAC CAT-3′). The primers for nested PCR were GeneRacer 5′ Nested Primer and *zfmstn2*-GSP2 (5′-CTG CAA AGA AAA TTG GGT TGG CCT TGT TG-3′). PCR amplification conditions were 35 cycles of 30 s at 94°C, 30 s at 55°C, and 2 min at 72°C. The PCR product was analyzed by 1% agarose gel electrophoresis. A discrete PCR product detected was isolated and subcloned into pGEM-T Easy cloning vector (Promega) and subjected to nucleotide sequencing (ABI). To obtain the 3′-coding region and 3′ untranslated region (3′ UTR) sequence further downstream, 3′ RACE was performed using the full length cDNA. PCR amplification and subcloning procedures were the same as above. Primers for first PCR were GeneRacer 3′ Primer and *zfmstn2*-GSP3 (5′-AGA CAC AGG GAA GCG ATC CAG AC-3′). The primers for nested PCR were GeneRacer 3′ Nested Primer and *zfmstn2*-GSP4 (5′-TTT CTT TGC AGG TCC CTG CTG TAT TCT-3′). The nucleotide and the deduced amino acid sequences of the cDNA were analyzed using a BLAST search for sequence homology. Multiple sequence alignment was performed using Pileup program in Unix-based GCG Wisconsin Package and illustrated using GeneDoc program (http://www.psc.edu/biomed/genedoc/). Phylogenetic analysis was conducted using a web-based sequence analysis program (SeqWeb Version2, Accelrys)

with Jukes-Cantor distance correction method. Neighbor-joining method and branch lengths proportional to distance were used to construct and display the tree, respectively.

To amplify the full-length zebrafish *mstn* cDNA, a set of sense and antisense primers (5′-GGA TCC ACC ATG TTT CTC CTT TTT TAT CTG AG-3′), (5′-GAA TTC AGA GCA ACC GCA AAG GTC TAC CAC C-3′) corresponding to the start and stop codon of the open reading frame (ORF), respectively, were synthesized. A PCR was carried out using the above primers, Platinum *Taq* DNA polymerase (Invitrogen) and zebrafish first-strand cDNA as the template. Amplification conditions were 35 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min and 30 s at 72°C. The PCR products were subjected to 1.5% agarose gel electrophoresis. The discrete PCR product band, visualized by ethidium bromide staining, was excised from the gel and purified using Gel Extraction Kit (QIAGEN). The purified zebrafish *mstn* cDNA was subcloned into pcDNA3.1 eukaryotic expression vector at *Bam*HI and *Eco*RI restriction sites. To verify its authenticity and direction of cloning, the cDNA insert was sequenced (ABI).

## RT-PCR analysis

First strand cDNA was synthesized from 5 μg of total RNA using Thermoscript™ RT-PCR system (Invitrogen). PCR reaction was performed with 2 μl of the RT reaction. The PCR program was as follows: one cycle 94°C, 30 s, 55°C for 30 s, and 72°C for 1 min and performed 30 cycles. The final extension time was 7 min at 72°C. The RT–PCR products were separated by 2% agarose gel electrophoresis. Max was used as an internal control.

## Whole mount in situ hybridization

Control and overexpressed embryos at different developmental stages were fixed, dechorionated and whole mount in situ hybridization was carried out by using dioxigenin (DIG) labeled riboprobe [25]. By T7 and SP6 RNA polymerase, the DIG labeled antisense and sense probes were synthesized. We carried out cryosection in 48 hpf embryos.

## Plasmid construction for overexpression

The full length of *mstn2* ORF was amplified and cloned into pcDNA3 eukaryotic expression vector at *Bam*HI and *Eco*RI restriction sites. The concentration of plasmid was determined by spectrophotometer and 20 μg/ml was injected into embryos between one cell and four cell stages.

## Immunohistochemistry

Immunohistochemisty was performed by using Anti-dystrophin MANDRA1 (Sigma), Anti-β-dystroglycan (Novocastra), β-sarcoglycan (Novacastra), anti-rabbit conjugated goat HRP (secondary) was diluted as 1:1,000, 1:100, 1:100, 1:1,000, respectively.

## Western blotting

Total protein extract was prepared using lysis buffer and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; 12%). For the separation of large proteins, Tris acetate gradient gels were used and transferred onto a nitrocellulose membrane. The membrane was blocked by net buffer at 4°C overnight and the bands were detected using the above antibodies. The membrane was washed, and the activity was detected using an ECL kit according to manufacturer's protocol.

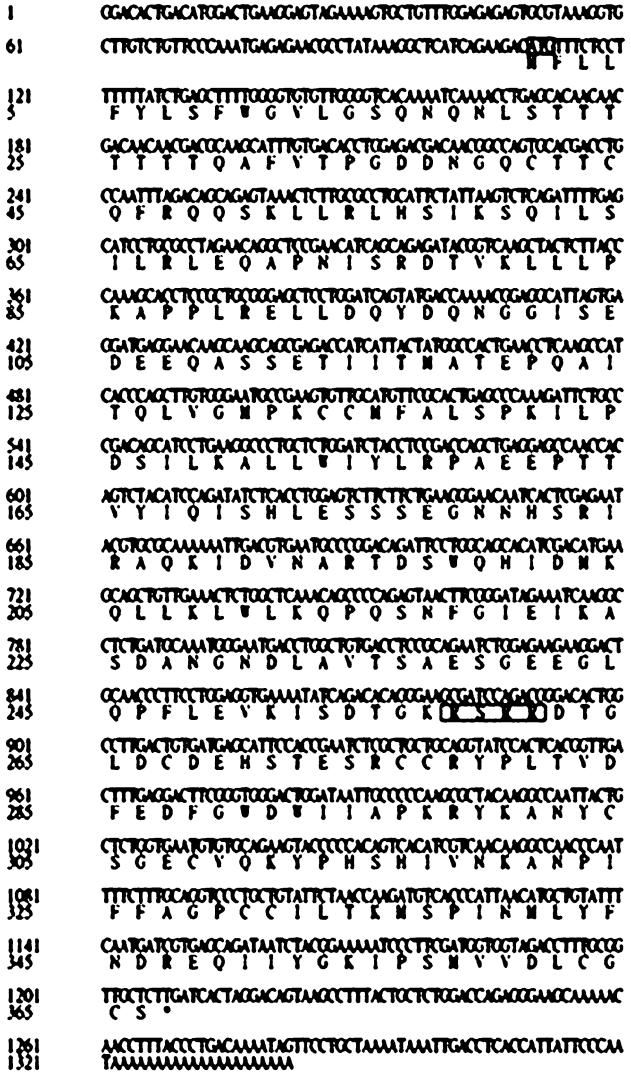

**Fig. 1** Nucleotide and deduced amino acid sequence of the zebrafish *mstn2* cDNA. Nucleotides are numbered 5′→3′. The translation start codon ATG is boxed and the asterisk indicates the translation stop codon. The putative proteolytic processing site (RSRR) is from R258 to R261. This sequence has deposited in GenBank™ (AY614000)

## Results

### Cloning and sequencing of zebrafish *mstn2* cDNA

Recently, we have isolated *mstn1* from zebrafish and demonstrated its function in regulating myogenesis during embryogenesis [6]. However, double muscle phenotype was not prominent as in mammals by the *mstn1* morpholino knock-down; moreover the tissue-distribution of zebrafish *mstn1* is not restricted to the skeletal muscle [6]. So we suspected that another form of *mstn* may exist in zebrafish and control the muscle growth as in the mammalian system. By in-silicon procedure to search potential candidate of Mstn isoforms from zebrafish genomic database, we identified one zebrafish genomic clone (zfish41364-181h04.q1c), which contained highly conserved C-terminal region of TGF-$\beta$ family protein using human cDNA as virtual probe. We designed gene-specific primer sets according to this candidate sequence and performed 5′ and 3′ RACE to obtain the full length of this cDNA using RNA ligase-mediated RACE (RLM-RACE). The size of the cDNA was 1342 bp with 109 and 132 bp

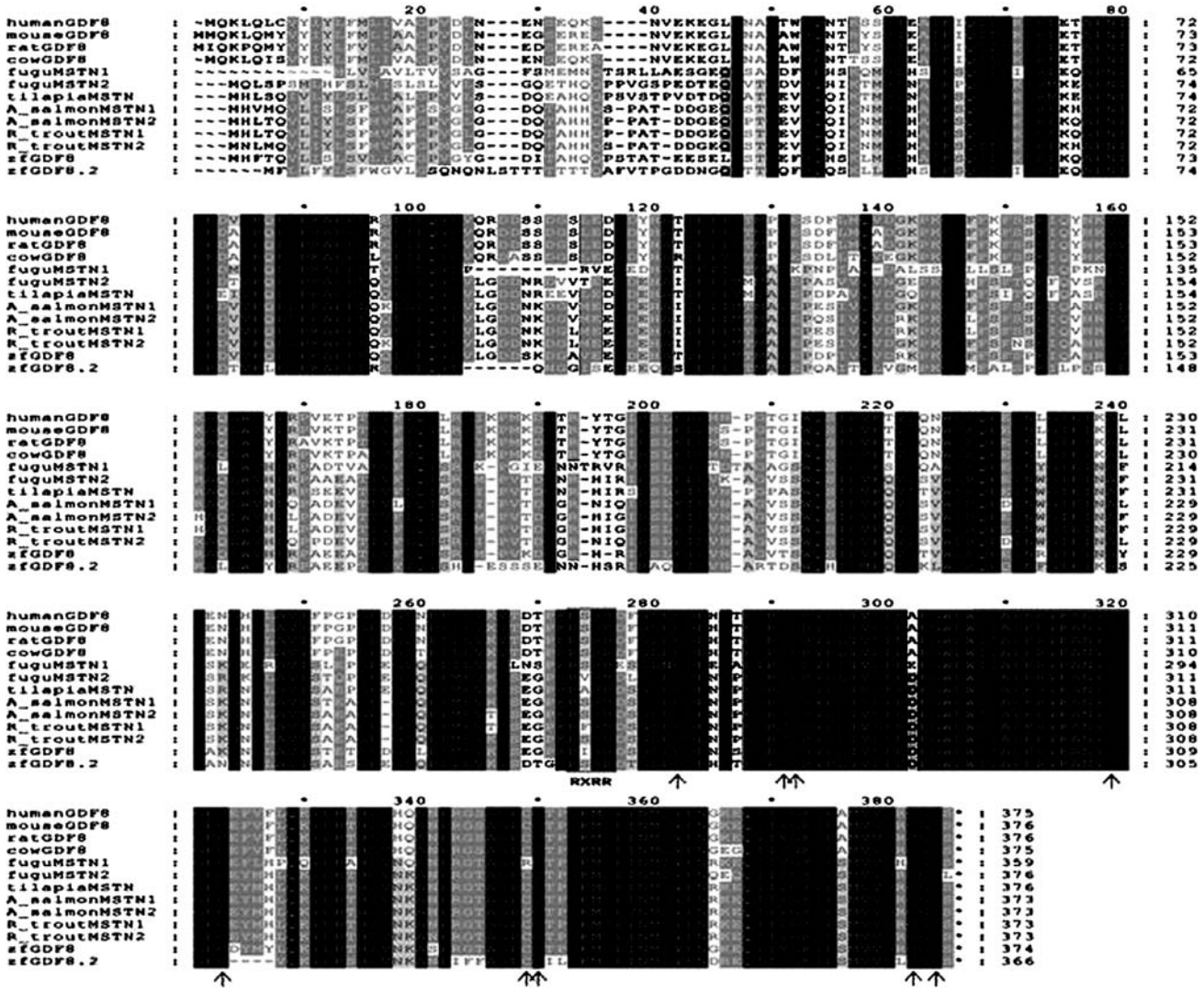


**Fig. 2** Comparison of the deduced amino acid sequences of zebrafish Mstn2 and myostatin from mammals and fish species. The nine conserved cysteine residues consisted in TGF-$\beta$ superfamily motifs are indicated with arrows. The potential proteolytic processing site (RXRR) is also boxed. The shading indicates the amino acids fully conserved among the species compared, and the gaps are shown as dashes (–). Sequences used in this comparison are deduced from human GDF8 (NM_005259), mouse GDF8 (NM_010834), rat GDF8 (NM_019151), cow GDF8 (AY160688), fugu Mstn1 (AY445321), fugu Mstn2 (AY445322), tilapia Mstn (AF197193), A_salmon Mstn1 (atlantic salmon; AJ297267), A_salmon Mstn2 (AJ344158), R trout Mstn1 (rainbow trout; AF273035), R trout Mstn2 (AF273036), and zMstn (zebrafish; NM_131019)

of 5′ and 3′ UTRs, respectively. The coding region is 1101 bp encoding 367 amino acids (Fig. 1). By BlastX search, this sequence was found to have high similarity with known *gdf8* from various species and with the highest score fit to zebrafish GDF8. This sequence is referred to as zebrafish *mstn2*. The nucleotide sequence identity between zebrafish *mstn1* and *mstn2* was only 66.9% which is far lower than other fish species. For example, the nucleotide sequence identity between *mstn1* and *mstn2* is 94.2%, 93.8% in Atlantic salmon and rainbow trout, respectively zebrafish *mstn2* is not considered to be a duplicated form of *mstn* because of their low identity at the coding and 3′ UTRs.

## Amino acid sequence homology and phylogenetic analysis

The zebrafish Mstn2 encodes a polypeptide consisting of 367 amino acids. Like all TGF-$\beta$ family, amino acid sequence homology analysis of the Mstn1 with Mstn2 from other species contain putative RXRR proteolytic processing site and nine conserved cysteine residues (Fig. 2). When we compared the zebrafish Mstn1 and Mstn2, the percentage of amino acid identity and similarity were 67.2% and 73.1%, respectively.

The phylogeneic relationship of zebrafish *mstn2* with other full length *gdf8* available in GenBank is depicted in

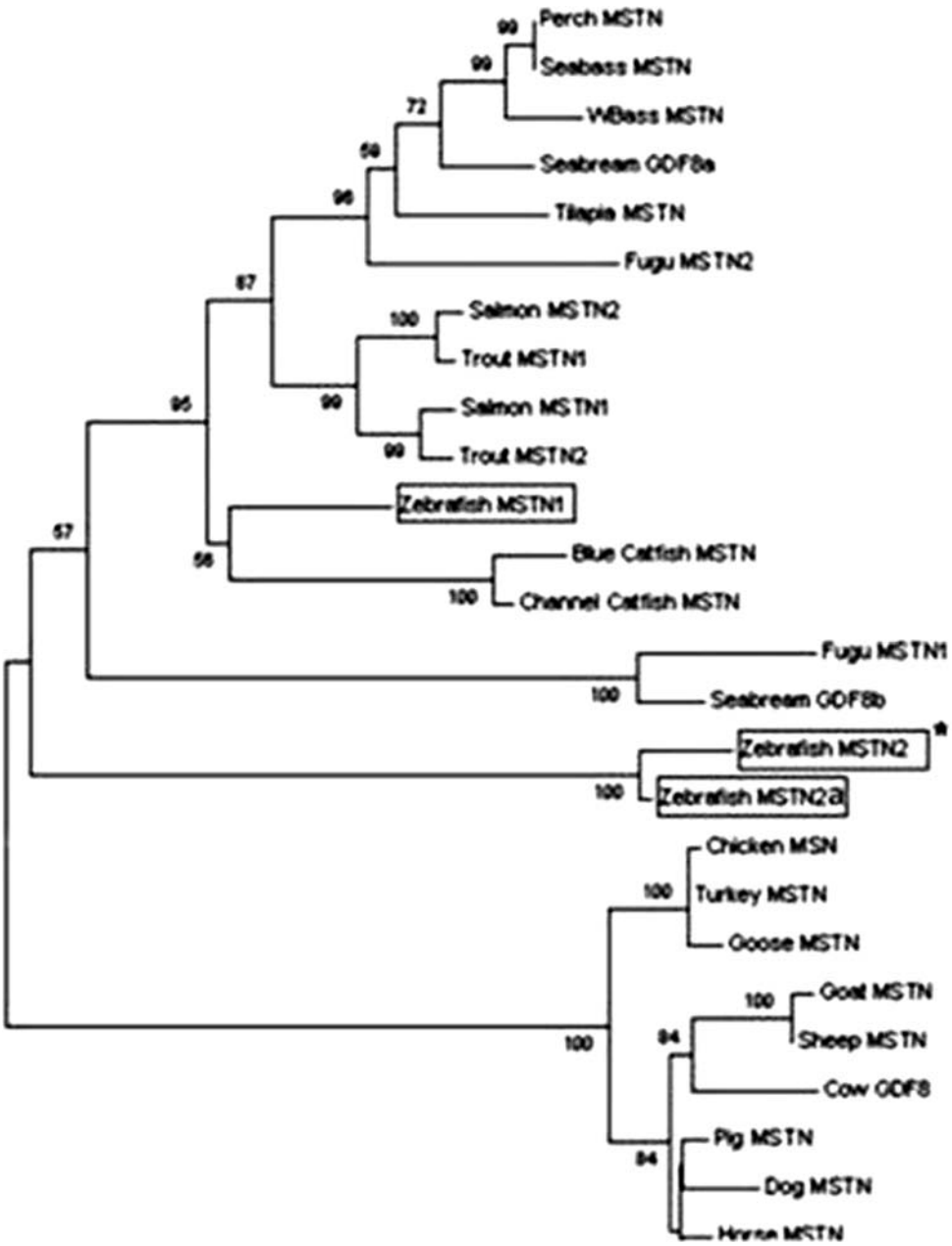


**Fig. 3** Phylogenetic tree of *mstn2*. The tree was built with known *mstn* sequences from the GenBank using the Neighbor-Joining method. Three gdf11 sequences were used as an out group. Sequences, in addition to those in Fig. 2, used in construction of this phylogenetic tree are baboon Mstn (hamadryas baboon; AF019619), goose Mstn (AY448009), turkey Mstn (AF019625), chicken Mstn (AY448007), dog Mstn (AY367768), pig Mstn (AY448008), horse Mstn (AB033541), goat Mstn (AY436347), sheep Mstn (AF019622), B catfish Mstn (blue catfish; AY540 992), C catfish Mstn (channel catfish; AF396747), G_seabreamGDF8 (gilthead seabream; AF258448), G_seabreamGDF8b (AY046314), S_seabass Mstn (striped sea-bass; AF290910), W_perch Mstn (white perch; AF290911), Whitebass Mstn (AF197194), human GDF11 (NM_005811), mouse GDF11 (XM_125935), rat GDF11 (XM_343148), and zfGDF11-like (zebrafish; AF411599)

Fig. 3. The result showed that zebrafish *mstn2* might be the ancestral form of *mstn1*.

## Mstn2 overexpression disrupts early muscle development

To understand whether *mstn2 is* related to muscle dystrophy, we overexpressed *mstn2*. Following injection of *mstn2* at one cell stage, embryogenesis proceeds normally. By 24 hpf, however, the embryos appeared to be developmentally delayed. Once the fish hatched out from the chorion (Fig. 4), the overexpressed embryos were curved or bent (Fig. 4b) and dystrophic compared with controls (Fig. 4a). The overexpressed embryos showed less motile and moved in an uncoordinated fashion. The cryosection data also showed distruption of myotomes and spaces in between the muscle fibers (Fig. 4d, f) when compared to the controls (Fig. 4c).

## Down-regulation of dystroglycogen complex (DGC) in zebrafish by overexpression of *mstn2*

Mstn2 overexpressed zebrafish embryos were analyzed at transcription and translational levels to determine the reduction of DGC (dystrophin, dystroglycan, and sarcoglycan) in the injected embryos. Among the markers, related to muscle dystrophy syndrome such as dystrophin, dystroglycan, sarcoglycan, calpain, caveolin and dysferlin, the expression of dystrophin, dystroglycan, and sarcoglycan were decreased.

RT-PCR results revealed that there was a reduction of dystrophin, dystroglycan and sarcoglycan (Fig. 5). The whole mount in situ hybridization of 12 and 96 hpf control embryos, revealed a wide spread distribution of mRNA in the somite (Fig. 6). In overexpressed embryos, however, we did not observe the strips (V shaped somites) and there was no signal in the somites presumably due to the disorganization of cells within the somites.

Immunohistochemical analysis using different antibodies showed strong expression of dystrophin, dystroglycan, and sarcoglycan predominantly at the myosepta in the control embryos (Fig. 7a, c, e) and no signal could be observed in the injected embryos (Fig. 7b, d, f). To confirm the immunohistochemistry results and to investigate the level of protein expression, Western blot analysis was performed. Protein extracts were prepared from uninjected and overexpressed zebrafish embryos, separated by SDS-PAGE, and subsequently transferred onto nitrocellulose membrane and detected. Antibodies showed marked reduction of DGC and the expression was very high in the overexpressed embryos than the control (Fig. 7b).

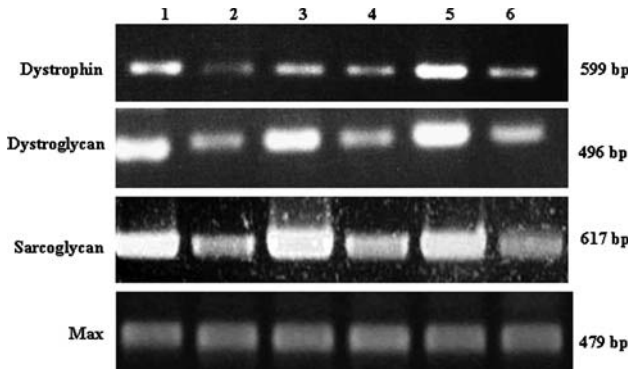


**Fig. 5** RT-PCR analysis of control and overexpressed embryos. 1, 3, and 5—12 h, 24 h, and 72 h control embryos; 2, 4, and 6—12 h, 24 h, and 72 h overexpressed embryos

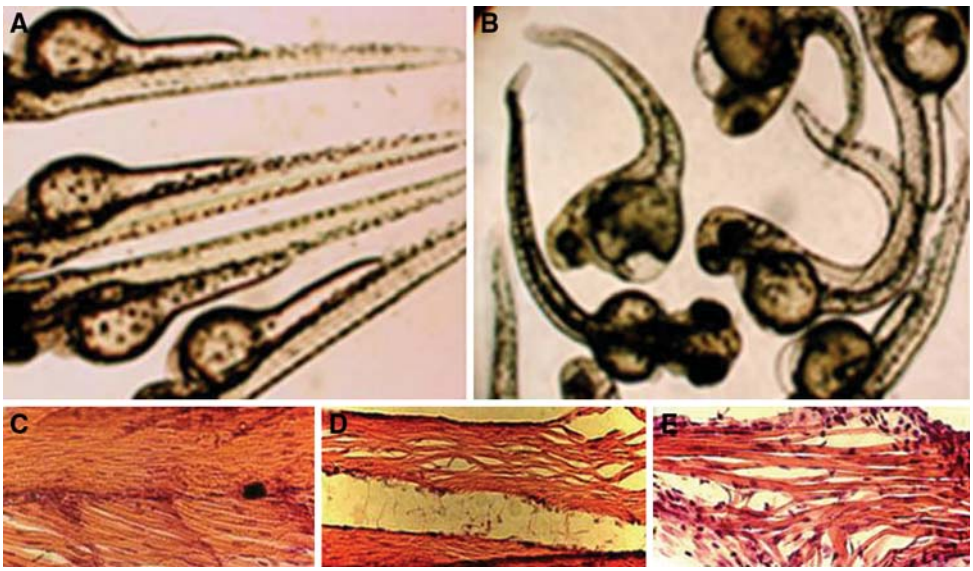


**Fig. 4** Zebrafish overexpressed with *mstn2* show curved and bent phenotypes. (**a**) 72 h control embryo, (**b**) 72 h overexpressed embryos, (**c**) cryosection of 72 h control embryo, and (**d** and **e**) cryosection of 72 h overexpressed embryos

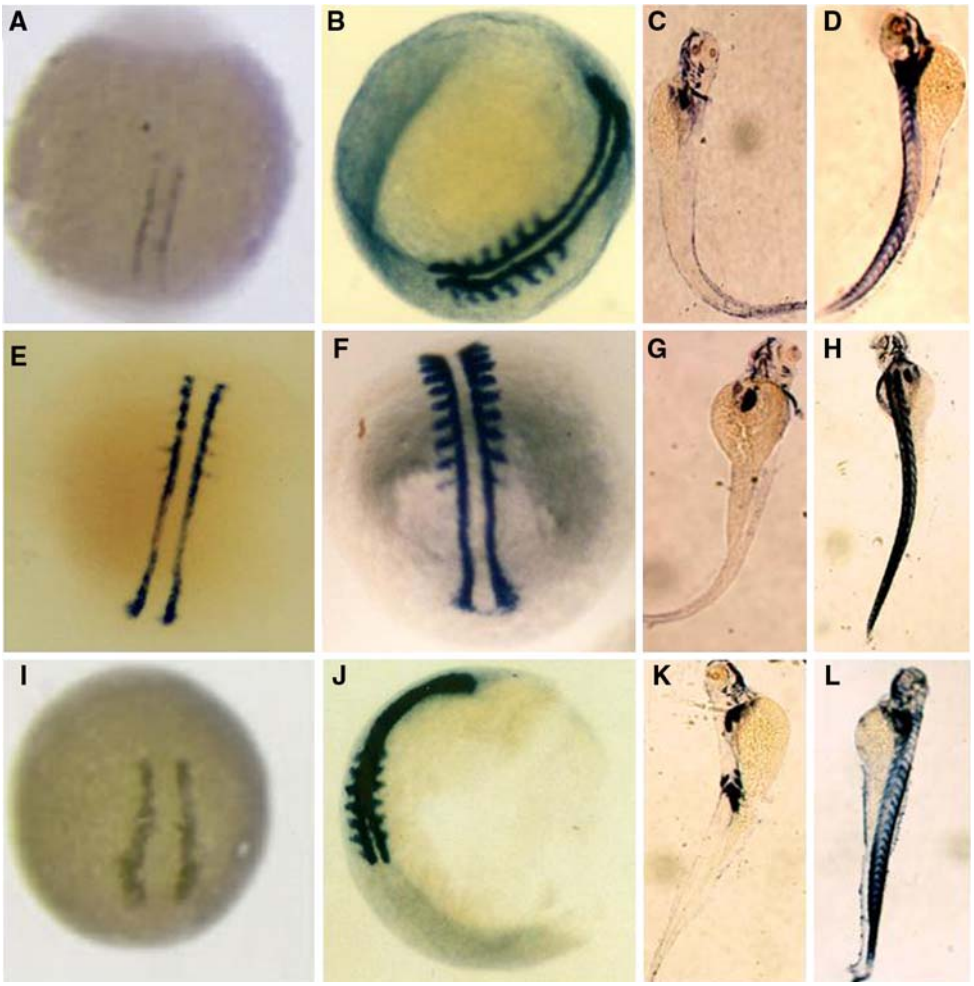


**Fig. 6** Whole mount in situ hybridization of control and overexpressed embryos. Embryos hybridized with dystrophin (**a–d**), dystroglycan (**e–h**), and sarcoglycan (**i–l**). (**a**, **e**, **i**) *mstn2* overexpressed 12 h embryos, (**b**, **f**, **j**) control 12 h embryos, (**c**, **g**, **k**) *mstn2* overexpressed 96 h embryos, and (**d**, **h**, **l**) control 96 h embryos

## Discussion

Here we report the identification and characterization of zebrafish *mstn2*. In mammals, myostatin plays a major role in muscle development. Though *mstn* has been cloned in different vertebrates, it is not known if *mstn2* has the same function in these animals as it does in higher vertebrates. The results of the present study clearly demonstrate several major differences between *mstn* expression in fish and other vertebrates. Comparison of nucleotide and amino acids showed very low percentage of similarity and identity. Phylogenetic tree analysis revealed that the zebrafish *mstn2* is an ancestral form. These results conclude that these two genes are derived from two different genes. In mice [5], cattle [26], and zebrafish [6], mutation or knockdown in the *mstn* gene showed a marked increase in body, muscle mass and growth. Inhibition of the *mstn* gene product is predicted to increase muscle mass and improve disease phenotype in a variety of primary and secondary myopathies [27]. Duchenne muscular dystrophy (DMD), the most common lethal muscle wasting disease is a result of an absence of muscle dystrophin. Although this disorder causes a rather uniform pattern of muscle wasting, affected patients display phenotypic variability. We hypothesized that genetic variation in myostatin is a modifier of the DMD phenotype [28]. The inhibition of *mstn* in vivo improves the dystrophic syndrome phenotype in the mdx mouse model of Duchenne muscular dystrophy (DMD) [29]. Transgenic expression of myostatin inhibitor follistatin increases the skeletal muscle and dystrophic pathology in mdx mice [30]. Blockade of endogenous Mstn by using intraperitoneal injections of anti-MSTN antibodies, for three months resulted in an increased body weight, muscle mass, muscle size and absolute muscle strength in mdx mouse along with a significant decrease in muscle degeneration and concentration of creatine kinase [27]. The more surprizing observation was the leakage of Mck into the serum, dropped to near wild-type levels in the anti-myostatin treated mice [31, 32]. It was proved that overexpression of *mstn* in mice leads to muscle loss [8, 9]. To study more on the functional aspects of *mstn1* and *2*, we knocked down *mstn1* [6] and overexpressed *mstn2* by CMV promoter. We observed the overexpressed embryos become less active, bent, and curved in the trunk region. The phenotypic difference is very much similar to muscle dystrophy

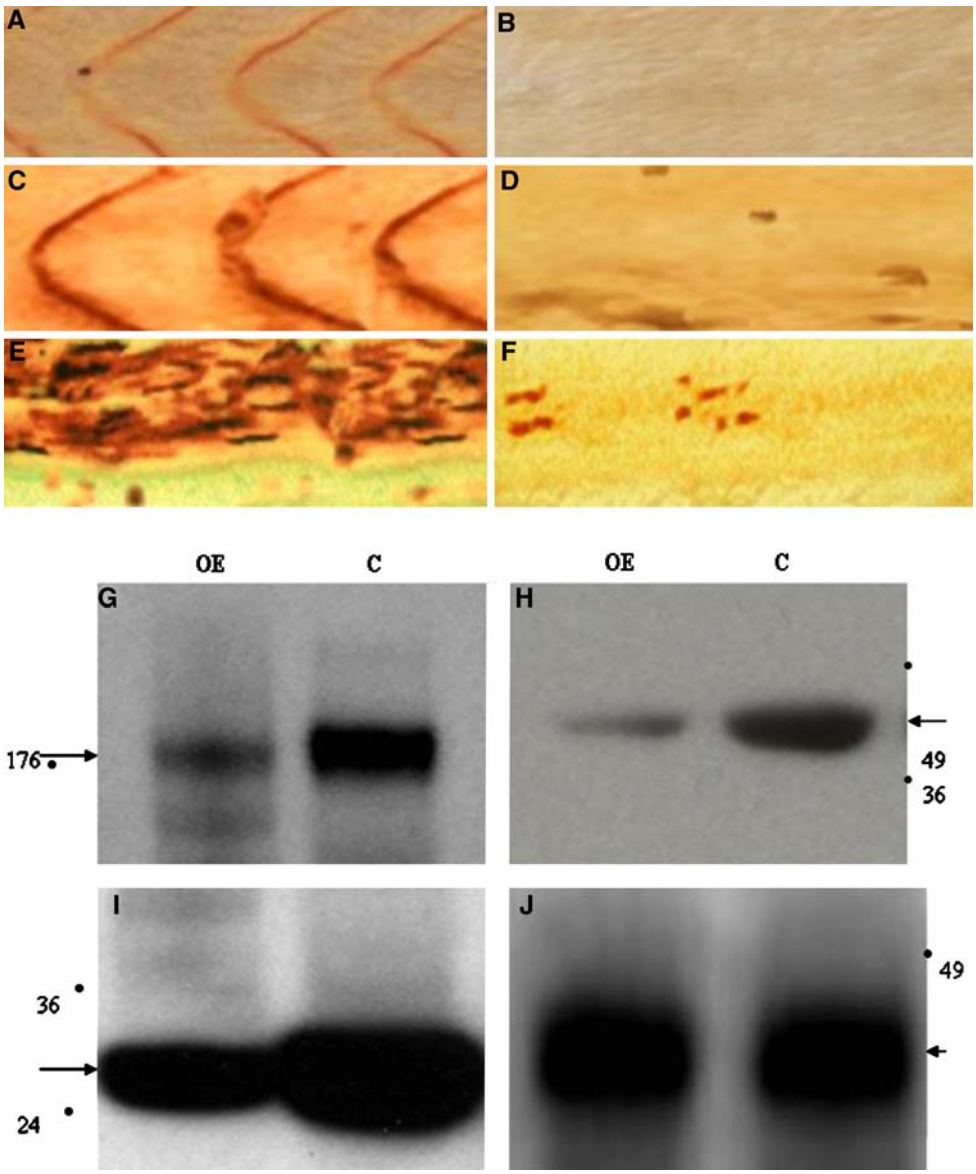


**Fig. 7** Immunohistochemical detection of control and overexpressed embryos by using Mandra1 (Anti-dystrophin antibody), anti-dystroglycan antibody, and sarcoglycan antibody. (**a**, **c,** and **e**) Control-96 h embryos, (**b**, **d,** and **f**) Mstn2 overexpressed 96 h embryos (**b**). Decreased (dystrophin, dystroglycan, and sarcoglycan) expression of and overexpressed embryos. Protein extracts from the 96 hpf injected with approximately 20 ng of *mstn2* plasmid and were separated by electrophoresis, transferred to a nitrocellulose membrane and analyzed by westernblotting. OE—Mstn2 overexpressed 96 h embryos. (**g**) Anti-dystrophin antibody, (**h**) anti-dystroglycan antibody (**i**) sarcoglycan antibody and (**j**) $\beta$ actin antibody

syndrome phenotype. To confirm the muscle dystrophy, we used different marker genes related to muscle dystrophy and checked at RNA and protein level (Figs. 6 and 7). The results showed decreased expression of dystrophin, dystroglycan, sarcoglycan. Our result was supported in the muscle dystrophy syndrome phenotype showed absence of dystrophin, dystroglycan, and abnormally high plasma and muscle creatine kinase [33, 34]. In vertebrate skeletal muscle, the DAPE is thought to link the extracellular matrix to the intracellular cytoskeleton [35]. Orthologs of proteins in the human DAPC have been identified in zebrafish [36–38]. Morpholino experiments directed against dystrophin and $\beta$-dystroglycan mRNA show both specific reduction of the dystrophin protein and repression of DAPE proteins [24, 38, 39]. In the *mstn2* overexpressed embryos, the phenotypic difference shared to varying extents with those found in zebrafish due to morpholino based translational knock down of dystrophin [38] and dystroglycan [24]. Myostatin mutations and pharmacological strategies increase muscle mass in vivo, suggesting that interruption of myostatin expression may be useful in diseases characterized by muscle wasting, such as the muscular dystrophies. It has been demonstrated in ϒ-sarcoglycan-deficient mouse model of limb-girdle muscular dystrophy (LGMD) 2C by antibody-mediated myostatin blockade in vivo, led to increased fiber size, muscle mass and absolute force [40]. Furthermore, systemic gene delivery of myostatin propeptide (MRPO), a natural inhibitor of myostatin, could enhance body-wide skeletal muscle growth [41].

To determine precisely the cellular basis for muscle degeneration, we continuously monitored mutant somites by cryosection. We observed that the disintegration of

somites, lack of transverse myoseptum and loss of muscle integrity due to muscle attachment failure were observed in the *mstn2* overexpressed embryos. The disruption is characterized by cheveron shaped myosepta yielding to U-shaped somites [42]. The transverse section revealed that the cellular appearance of muscles in overexpressed embryos was less organized than the control embryos. The mode of muscle cell loss in overexpressed embryos is similar to the loss of muscle in human congenital muscular dystrophy in several ways. Through histological studies, we have shown that the overexpression of *mstn2* leads to loss or mislocalization of somites with a general disruption of muscle integrity of the muscle that may be due to the loss of dystroglycan, dystrophin and sarcoglycan because they are important for muscle integrity [43, 32]. These phenotypes are very similar to those observed in muscle degeneration that occurs in human suffering from muscular dystrophy. Translation blocking morpholinos targeted to a zebrafish dystrophin was recently reported to cause an uncharacterized disorganization of the somites similar to that seen in degenerating *sap* mutant embryos, and reduced levels of the DAPC component $\beta$-sarcoglycan, although no specific defect in muscle fiber integrity was reported [24].

There are several features of zebrafish embryogenesis and genetics that make it well suited for the study of muscular dystrophy. Zebrafish embryonic skeletal muscle is simply organized with single myotubes extending across each somite and attached at either end to the ECM of the transverse myoseptum. Since dystrophin and dystroglycan are both expressed at early stages of zebrafish development [37], it is possible that the loss of these proteins could destabilize muscle fibers interaction at this location during critical stage of development [24].

Our data provide the first in vivo evidence of muscle attachment failure by the overexpression of *mstn2* which leads to muscle loss and muscle dystrophy that may contribute to Duchenne and other muscle diseases. Collectively, these results provide the first in vivo genetic evidence that *mstn* is not only important for myogenesis but if it is high it will collapse the vertebrate muscle attachments, as has been found in *mdx* mice, but also for their stability, and that failure of these attachments can lead to a progressive muscular dystrophy. Finally *mstn2* overexpression therefore provides a model for the novel pathological mechanism of muscle attachment failure that could well contribute to some of the many different human muscular dystrophies or congenital myopathies.

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