Myostatin Gene Editing Across Vertebrates, Comparative Insights from CRISPR, TALENs, and AAV-Mediated Strategies

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Abstract

Myostatin (MSTN/GDF-8) is a conserved negative regulator of skeletal muscle growth in vertebrates. Recent advances in genome-editing technologies, including CRISPR/Cas9, TALENs, and AAV-mediated approaches, have enabled precise MSTN knockout across mammals, fish, and avian species. MSTN inhibition consistently enhances muscle mass through hyperplasia and hypertrophy, elevates myogenic transcription factors such as MyoD and Myf5, and modulates metabolic pathways, including glucose homeostasis and adipose deposition. MSTN knockout also affects systemic physiology, including the gut–muscle axis, bone density, and neuromuscular function, revealing pleiotropic effects beyond muscle growth. Comparative studies in rabbits, pigs, channel catfish, zebrafish, chickens, and mice demonstrate species-specific outcomes, highlighting the importance of tailored strategies for therapeutic or agricultural applications. While MSTN-targeted interventions show promise for muscle-wasting disorders, sarcopenia, Duchenne muscular dystrophy, and cultured-meat production, challenges remain, including off-target effects, reproductive and skeletal trade-offs, and limited clinical translation. This review synthesizes recent research on MSTN knockout, emphasizing conserved mechanisms, phenotypic consequences, and translational potential, providing a framework for future studies aimed at safely exploiting MSTN inhibition to enhance muscle mass and metabolic health.

Keywords: Myostatin, MSTN knockout, CRISPR/Cas9, TALENs, muscle hypertrophy, vertebrates, gene editing, metabolic regulation, myogenic transcription factors, cultured meat.

Introduction

Skeletal muscle development is tightly regulated by a balance of anabolic and catabolic signals, with myostatin (MSTN/GDF-8) functioning as a master negative regulator of muscle growth. Loss-of-function mutations in MSTN lead to remarkable muscle hypertrophy across species, making it a prime target for both therapeutic and agricultural applications. Recent progress in gene-editing technologies, particularly CRISPR/Cas9, has revolutionized MSTN research, enabling precise, efficient, and species-specific disruption of the gene. MSTN knockout models in mammals, fish, and avian species have revealed not only enhanced muscle mass but also alterations in metabolism, satellite-cell activity, bone density, and gut microbiota composition. These findings underscore the multifaceted roles of MSTN in muscle, metabolic, and systemic physiology, offering insights for Duchenne muscular dystrophy treatment, sarcopenia, livestock improvement, and cultured-meat production. This review integrates recent

experimental findings to highlight conserved mechanisms, phenotypic outcomes, and translationa potential of MSTN inhibition.

Literature Review

CRISPR/Cas9 was used to generate MSTN-knockout rabbits by targeting two sgRNAs to the coding region of the MSTN gene. Cas9 mRNA and sgRNAs were microinjected into rabbit zygotes, and analysis of 12 resulting blastocysts showed that 10 (83.3%) carried MSTN mutations, including 3 (25%) with biallelic edits, demonstrating high genome-editing efficiency. To obtain knockout animals, 158 edited embryos were transferred into four surrogate mothers, producing 20 live pups. Genotyping confirmed MSTN mutations in 16 of these pups, with indels ranging from 3 to 76 bp, including frequent large fragment deletions between sgRNA target sites. By four months of age, F0 MSTN-KO rabbits displayed the characteristic double-muscle phenotype. Off-target analysis of predicted sites revealed no detectable mutations, indicating high specificity of the CRISPR/Cas9 system used in this study [1].

Researchers also used TALENs to knock out the mstnb gene in zebrafish by targeting exon 1. Gene editing was verified by AseI digestion, and two mutant lines were established—one carrying a small deletion and the other an insertion. Both mutations caused premature translational termination, producing truncated, non-functional MSTNb protein. Importantly, there was no compensatory upregulation of mstna or mstnb in the mutants. As the fish grew, they became ~21% heavier than wild-type animals at 80 days post-fertilization, while body length remained unchanged, indicating that increased muscle mass was the primary contributor to weight gain. Histological analysis showed more numerous but smaller muscle fibers, demonstrating hyperplasia rather than hypertrophy—a phenotype that differs from mammalian MSTN knockouts. Expression of myogenic regulators such as myoD, myoG, and Myf5 was significantly elevated, while satellite-cell markers (pax3, pax7, Mrf4) were unchanged. Metabolic changes included elevated blood glucose and increased intramuscular adipose deposition, similar to MSTN-deficient mice[2].

Efficient CRISPR/Cas9-mediated myostatin editing in pigs has also been reported. Wang et al. demonstrated that optimization of electroporation parameters greatly improved transfection in porcine primary fibroblasts, achieving ~90% efficiency—significantly higher than with FuGENE HD. Using the optimized protocol, CRISPR/Cas9 targeting of MSTN produced mutation rates of 12–22% with individual sgRNAs. Dual-sgRNA delivery increased editing efficiency to ~31% and generated large deletions and inversions between the target sites, an approach also used successfully in other MSTN-editing studies. ssDNA-mediated HDR successfully introduced a 4-bp insertion, although at low efficiency. Collectively, the optimized electroporation strategy significantly improved MSTN gene editing in pigs [3].

CRISPR/Cas9-mediated disruption of MSTN in channel catfish was accomplished by microinjecting Cas9 protein along with three sgRNAs (MSTN-1, MSTN-2, MSTN-3) or a multiplex mixture targeting exon 1. All sgRNAs exhibited strong on-target cleavage activity, producing very high mutagenesis efficiencies (88–100%). The multiplex treatment generated the most diverse indels, including large deletions (153–316 bp) that removed much of exon 1. Most mutations were frameshifts predicted to produce truncated MSTN protein. Although embryo mortality increased due to microinjection stress, hatchability (25–57%) and fry survival (85–100%) remained high, with no developmental abnormalities observed. Nearly all dead embryos were mutated, indicating efficient one-cell-stage editing. Mutant fry exhibited ~29.7% higher body weight and ~6.6% longer body length than wild-type at 40 days post-fertilization, confirming MSTN's conserved role as a negative regulator of muscle growth [4].

Recent work in large mammals shows that MSTN knockout also affects the gut–muscle axis. In MSTN−/− pigs, skeletal muscles were significantly enlarged, especially type IIb fast-twitch fibers, with elevated MyHC-IIb, MyoD, and glycolytic enzymes and reduced Smad2/3 phosphorylation. Interestingly, MSTN deletion reshaped the gut microbiota, increasing short-chain-fatty-acid-producing bacteria. Fecal microbiota transplants from MSTN−/− pigs into normal mice resulted in larger gastrocnemius muscles, hypertrophic type IIb fibers, and activation of the Akt/mTOR pathway. These mice showed increased grip strength and reduced endurance, indicating enhanced

fast-twitch glycolytic muscle development. This work demonstrates that MSTN knockout promotes muscle growth both directly and indirectly via gut microbial modulation [5].

Additionally, MSTN knockout potentiates the activity of key myogenic transcription factors. Eom et al. showed that in bovine fibroblasts, MSTN-deficient cells displayed enhanced responsiveness to MyoD1-mediated myogenic conversion. MyoD1 overexpression was confirmed by qRT-PCR and immunofluorescence, and MSTN-KO cells formed well-aligned myotubes in a 3D bioprinting system. This highlights the synergistic interplay between MSTN inhibition and MyoD1 activation, offering significant potential for cultured-meat production and therapeutic muscle regeneration [6, 7].

Recent advances in zebrafish genome engineering directly strengthen MSTN knockout research. The review on expanding the CRISPR toolbox shows how technologies such as base editing, CRISPRi/CRISPRa, and inducible or tissue-specific systems now allow finer control of mstn/mstnb regulation beyond simple knockouts [8]. The CRISPR-based genome editing overview reinforces why zebrafish are ideal for MSTN studies—rapid development, transparent embryos, human gene homology—and highlights knockout/knock-in applications relevant for modeling muscle-related phenotypes [9]. The muscular dystrophy modeling review demonstrates how mutant lines and fluorescent biosensors enable real-time visualization of muscle damage and regeneration, tools highly valuable for assessing hypertrophy or compensatory effects in MSTN-deficient zebrafish [10]. Finally, the gene-editing technology progress review explains the shift from ZFNs/TALENs to highly efficient CRISPR-Cas platforms, supporting rapid generation of mstn knockout lines and multiplex editing strategies for studying muscle growth mechanisms [11].

Adenoviral CRISPR/Cas9 editing in chickens produced large MSTN deletions that significantly reduced gene expression and activated muscle-growth pathways such as PDGF and STAT3, resulting in enhanced muscle development [12]. Likewise, TALEN-mediated mstnb depletion in zebrafish increased body size and muscle hyperplasia while upregulating key myogenic regulators including myod and myog, confirming MSTN's conserved role as a negative regulator of muscle growth [13]. Supporting these findings, AAV–SaCas9-mediated Mstn editing in aged mice effectively knocked down MSTN, enlarged muscle fibers, increased satellite cell activity, activated AKT/mTOR signaling, and reduced muscle-atrophy markers such as MuRF1 and MAFbx [14]. Collectively, these studies show that MSTN suppression—via CRISPR, TALENs, or AAV–SaCas9—consistently enhances muscle growth across vertebrate models by promoting anabolic signaling and reducing catabolic pathways.

Myostatin (MSTN/GDF-8) is a key negative regulator of skeletal muscle growth, and its inhibition is being explored as a therapeutic approach to enhance muscle mass and metabolic health (15). Several strategies—such as monoclonal antibodies, soluble receptor traps, propeptide blockers, and ligand-neutralizing molecules—have produced marked muscle hypertrophy and strength gains in animal models. Myostatin inhibition has shown potential benefits in Duchenne muscular dystrophy, sarcopenia, cachexia, obesity, insulin resistance, and bone-related disorders, improving muscle mass, reducing fat, and enhancing glucose metabolism and bone regeneration (16). However, clinical translation is limited by low myostatin levels in humans, off-target effects on TGF-β family members, poor inhibitor specificity, and weak correlation between muscle growth and functional outcomes. Future progress will require highly specific inhibitors, better patient selection, combined therapeutic strategies, and broader evaluation in metabolic and orthopedic conditions.

Augustin et al. (2017) demonstrate that the Drosophila myostatin homolog Myoglianin (MYO) regulates both muscle growth and neuronal connectivity. MYO reduction strengthens NMJ transmission, increases bouton number and glutamate receptors, and improves locomotion, whereas MYO overexpression causes smaller muscles and weakened synaptic function. MYO also shapes central neural circuits, and similar inhibitory effects of Myostatin/GDF11 on neurite growth in mammalian neurons suggest conserved TGF-β/Smad2–Akt/GSK3 signaling. These findings reveal MYO's dual muscle–neuronal regulatory role with implications for neuromuscular disease and gene therapy. [17]

Recent advances in gene-editing have expanded the understanding of myostatin's conserved role in regulating muscle mass across vertebrate species. In mice, CRISPR-mediated disruption of Mstn resulted in pronounced skeletal muscle hypertrophy, reduced intramuscular fat, and improved metabolic profiles, demonstrating systemic benefits of MSTN loss beyond muscle enlargement [18]. Similarly, MSTN-knockout pigs produced via zygote injection showed greater muscle mass and enhanced feed efficiency, although some lines displayed reproductive abnormalities, highlighting important species-specific physiological consequences [19]. In zebrafish, targeted deletion of mstnb significantly increased somatic growth, myofiber size, and swimming performance, reinforcing the fundamental evolutionary role of myostatin in restricting vertebrate muscle development [20]. Rabbit models further confirmed these trends, where MSTN-null animals exhibited dramatic skeletal muscle expansion but concurrently showed reduced bone density, pointing to a mechanistic link between muscle hypertrophy and skeletal adaptation [21]. Expanding these insights to avian species, adenovirus-delivered CRISPR targeting of MSTN in chickens led to increased muscle fiber diameter and elevated expression of myogenic markers, demonstrating the feasibility of viral MSTN-editing for agricultural applications [22]. Together, these studies show that MSTN knockout consistently enhances muscle mass across species while also revealing important metabolic, skeletal, and reproductive trade-offs that must be carefully considered when designing therapeutic or livestock-oriented myostatin-editing strategies.

Conclusion:

MSTN knockout consistently promotes skeletal muscle growth across vertebrate species, primarily through hyperplasia and hypertrophy, while modulating myogenic transcription factors and metabolic pathways. Genome-editing platforms such as CRISPR/Cas9, TALENs, and AAV-mediated strategies allow efficient and precise MSTN disruption, enabling mechanistic studies and applications in therapy, livestock, and cellular agriculture. Despite these advances, species-specific physiological consequences-including reproductive, skeletal, and metabolic trade-offs-require careful consideration. Future research should focus on optimizing gene-editing specificity, evaluating systemic effects, and integrating MSTN modulation with other anabolic or metabolic interventions. Collectively, these studies reinforce MSTN as a critical regulator of muscle mass and a promising target for translational and agricultural strategies.

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