

How to Cite:

Widjaja, J. S., Adji, A. S., Wardani, V. A. K., Santoso, E. L., Sunarto, F. R., Handajani, F., & Rahman, F. S. (2022). DMD, RIPK3, and MLKL gene editing by CRISPR Cas9 as myofiber protection against dystrophin deficiency and necroptosis in Duchenne muscular dystrophy: A literature review. *International Journal of Health Sciences*, 6(S6), 2199–2222. <https://doi.org/10.53730/ijhs.v6nS6.10886>

DMD, RIPK3, and MLKL gene editing by CRISPR Cas9 as myofiber protection against dystrophin deficiency and necroptosis in Duchenne muscular dystrophy: A literature review

Jordan Steven Widjaja

Medical Faculty of Hang Tuah University, Surabaya, Indonesia
Email: jordanstevenwidjaja20190410063@gmail.com

Arga Setyo Adji

Medical Faculty of Hang Tuah University, Surabaya, Indonesia
Email: argasetyo27@gmail.com

Vira Aulia Kusuma Wardani

Medical Faculty of Hang Tuah University, Surabaya, Indonesia

Eilien Levina Santoso

Medical Faculty of Hang Tuah University, Surabaya, Indonesia
Email: eilienlevinas20190410034@gmail.com

Fadhilla Rachmawati Sunarto

Medical Faculty of Hang Tuah University, Surabaya, Indonesia
Email: fadhillar.s.20190410036@gmail.com

Fitri Handajani

Biochemistry Laboratory Medical Faculty of Hang Tuah University, Surabaya, Indonesia

*Corresponding author email: fitrihandajanidr@gmail.com

Firman Suryadi Rahman

Abstract--BACKGROUND: Duchenne muscular dystrophy is a neuromuscular disease caused by a deficiency of dystrophin, which causes the skeletal and cardiac muscles to degenerate. Targeted deletion of DMD, RIPK3, and MLKL has been shown in several studies to prevent dystrophin deficiency and necroptosis, a critical hypothesis in the etiology of Duchenne muscular dystrophy. AIM: This research aimed to see if using CRISPR/Cas9 to target DMD, RIPK3, and MLKL

is an effective therapeutic and if it has a long-term effect on Duchenne muscular dystrophy. **METHODS:** Abstracts and titles of articles were searched for specific keywords to summarize them using the method used in this study. The researcher will look over the entire article to see if it is valuable and relevant to the topic. **RESULTS:** CRISPR/Cas9-mediated genome editing in MDX mice can improve the primary genetic lesions that cause muscular dystrophy (DMD) and prevent disease development. Furthermore, Ripk3/Mlk1 double knockout completely blocked necroptosis susceptibility in necroptosis-sensitive cell lines, each to an indistinguishable degree. **CONCLUSION:** DMD, RIPK3, and MLKL gene editing by CRISPR/Cas9 is effective dystrophin insufficiency, sarcolemma fragility, poor intracellular signaling, myocyte death, inflammatory infiltration, muscle replacement, and necroptosis. However, more research is needed to determine its side effects and safety.

Keywords---duchenne muscular dystrophy, CRISPR-Cas9, DMD, RIPK3, MLKL.

Introduction

One of the most damaging forms of inherited muscular dystrophy is Duchenne muscular dystrophy (DMD). Neuromuscular disease is the most common inherited condition in children, regardless of race or ethnicity. Muscle fibers degenerate and weaken due to mutations in the dystrophin gene. This weakness may begin with difficulty walking and progress to the point where people cannot perform daily activities and rely on a wheelchair. Cardiac and orthopedic problems are common, and respiratory muscle weakness or cardiomyopathy are the leading causes of death in people in their twenties. Glucocorticoids and physical therapy are the mainstays of current treatment (Bello & Pegoraro, 2019)(Tomar et al., 2019)(Paquin et al., 2019).

The most frequent kind of muscular dystrophy is Duchenne muscular dystrophy. The condition affects 15.9 to 19.5 live births out of every 100,000. DMD has a global frequency ranging from 0.1 to 1.8 per 10,000 males. Among the 10,000 men in South Africa, Asia, North America, and Europe, the prevalence ranges from 0.10 to 2.04 per 10,000 men, according to research (Ryder et al., 2017), (Romitti et al., 2015). Epidemiological data for DMD in Indonesia is currently scarce. However, a 2017 research at Cipto Mangunkusumo Hospital in Jakarta identified 179 people suspected of having neuromuscular problems. One hundred thirty individuals satisfied the criteria for neuromuscular diseases after completing an electromyography evaluation. DMD was recognized in 16 (12.3 percent) of the 130 individuals. Males are found to have a greater prevalence than females (Milanti Dewi, Putro Widodo, Amardiyanto, Sinaga, & Hidayah, 2018).

DMD is a genetic condition that affects the X chromosome Xp21 region, essential for creating the dystrophin protein. X-linked inheritance is defined by fathers' ability to pass on X-linked features to their sons. Men with DMD receive the gene

mutation from a mother who carries one copy of the gene in around two-thirds of cases. A third could result from a novel mutation in this gene (Cornelis & Gessal, 2021)(Vijay Venugopal; Steven Pavlakis, 2021). DMD symptoms in newborns, such as poor motor development, do not manifest until they reach the age of three. Patients between the ages of 8 and 10 find it difficult to stand and walk, necessitating the need for assistance or a wheelchair (Yao et al., 2021)(Birnkranz et al., 2018).

Duchenne and Becker's muscular dystrophy and the transitional type are caused by dystrophinopathies resulting from mutations in the dystrophin gene. Only trace amounts of the protein dystrophin are produced due to the transformation, resulting in sarcolemmal integrity loss and necrosis-regeneration cycles. Muscle is gradually replaced by fibrous connective tissue and fat, resulting in clinical manifestations (Falzarano, Scotton, Passarelli, & Ferlini, 2015). Some of the treatments for DMD that have been discovered are listed below. Corticosteroids, particularly prednisone and desflurane, are the most common drugs to treat DMD. Both deflazacort and prednisone were given 0.75 mg/kg daily or 10 mg/kg weekly for two weeks. Therapy with ACE inhibitors is recommended to prevent heart failure due to DMD. There are three approaches to exon skipping: oligonucleotides, CRISPR, and splice blocking mediated by U7 snRNP. For oligonucleotides, there are 2 AOs (Antisense Oligonucleotide) currently studied, namely 2'-O-methyl-phosphorothioates (2OMP) at exon 23 and Phosphorodiamidate morpholino oligomers (PMOs) at exon 51 (De Los Angeles Beytía, Vry, & Kirschner, 2012)(Takeda, Clemens, & Hoffman, 2021).

Myoblasts and satellite cells, myeloid cells and mesoangioblasts, and CD133+ cells have been studied as stem cell sources for DMD. Many other DMD therapies include utrophin, vitamin D supplements, myostatin, etc. However, DMD therapy has been found for a long time; until now, DMD therapy has not been found to completely cure DMD (Sun, Serra, Lee, & Wagner, 2020). R. Gupta, S. Sinharoy, K. Acharya, and D. Ghosh stated that “so far there are three technologies targeting gene editing: transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and short interspersed palindromic reps. -CRISPR-associated 9 (CRISPR-Cas9)” (Gupta, Sinharoy, Acharya, & Ghosh, 2019). CRISPR-Cas9 can target multiple genes because the protein remains the same for all. TALENs (transcription activator-like effector nucleases) and zinc finger nucleases (ZFNs) are less sophisticated gene editing and regulation alternatives. Site-specific fission is redirected by a short guide RNA sequence (Janik, Niemcewicz, Ceremuga, & Krzowski, n.d.). CRISPR/Cas 9 is a powerful but particular and efficient gene-editing system that can scan the entire genome accurately and quickly, making gene therapy for specific diseases easier to deliver. It is at least as effective as other methods while being less cytotoxic (Jiang, Meng, Yang, & Luo, n.d.), (Doetschman & Georgieva, 2017).

Materials and Methods

Google Scholar, Cochrane, Science Direct, PubMed, and Frontiers in Neuroscience were used to search for articles for this study. It was used in DMD searches, RIPK3, CRISPR-Cas9 genome editing, and regularly clustered MLKL punctuated short palindromic repeats. At the same time, articles were screened by

customizing the title and abstract to match the study's focus. Verify that the title and abstract match the full text of the article. If an article appears to be relevant and valuable, it is read entirely by the researcher.

Results and Discussions

Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is the most common form of the disease, affecting one in every 5,000 boys and presenting as proximal muscle weakness in the early stages of development in children (Moat, Bradley, Salmon, Clarke, & Hartley, 2013). Dystrophin deficiency causes skeletal and cardiac muscle degeneration, which leads to progressive muscular weakness and wasting (Falzarano et al., 2015). In affected boys, DMD manifests itself as proximal muscular weakness and calf enlargement in early childhood. Motor development is typically delayed, leading to wheelchair confinement and premature death from cardiac or pulmonary issues (Sinha, Sarkar, Khaitan, & Dutta, 2017). J. K. Mah revealed that “allele-specific mutations in the DMD gene create Duchenne muscular dystrophy (OMIM *310200) and Becker muscular dystrophy (OMIM *300376), a milder form of the disease” (Mah, 2016).

Dystrophin is a 427kDa cytoskeletal protein concentrated at costameres in muscle fibers and localizes to the cytoplasmic face of the sarcolemma (Porter, Dmytrenko, Winkelmann, & Bloch, 1992). DMD mutations result in a dystrophin protein that is prematurely truncated and unstable (Aartsma-Rus, Van Deutekom, Fokkema, Van Ommen, & Den Dunnen, 2006). Sarcolemma fragility, poor intracellular signaling, muscle cell death, inflammation, and eventually muscle replacement by fibrotic and fatty tissue are all consequences of dystrophin deficiency in skeletal muscle (Gao & McNally, 2015). Chang, Chevalier, and Rudnicki suggested that “satellite cells, an injury-responsive stem cell population underlying the basal muscle lamina, get activated and fuse with damaged myofibers as DMD progresses, eventually depleting this regenerative cell population and causing muscle degeneration” (Chang, Chevalier, & Rudnicki, 2016). On the other hand, the adult heart lacks a substantial stem cell population; therefore, dystrophin deficiency in the heart leads to myocyte malfunction, loss of pump function, and fatal cardiomyopathy (Fayssoil, Nardi, Orlikowski, & Annane, 2010).

Mitochondria mediate cell membrane repair and contribute to Duchenne muscular dystrophy

The poor sarcolemmal repair was revealed as an early consequence of dystrophin deficiency during our examination of early events during illness due to dystrophin deficiency. We present a model to explain the root cause of the poor repair (Figure 1). Calcium overload in dystrophic muscle occurs due to sarcolemmal tears generated by the dystrophin-deficient sarcolemma fragility and calcium leak channels. Mitochondria buffer part of the calcium excess. Autophagy causes a reduction in mitochondrial function as well as mitochondrial loss. Myofiber healing is hampered by mitochondrial dysfunction and loss, which raises calcium excess and sets up a positive feedback loop for dystrophic myofiber necrosis (Figure 1) (McCarter & Steinhardt, 2000)(Fong, Turner, Denetclaw, & Steinhardt,

1990)(Ng, Metzger, Claflin, & Faulkner, 2008)(Yeung et al., 2005)(Vila et al., 2017).

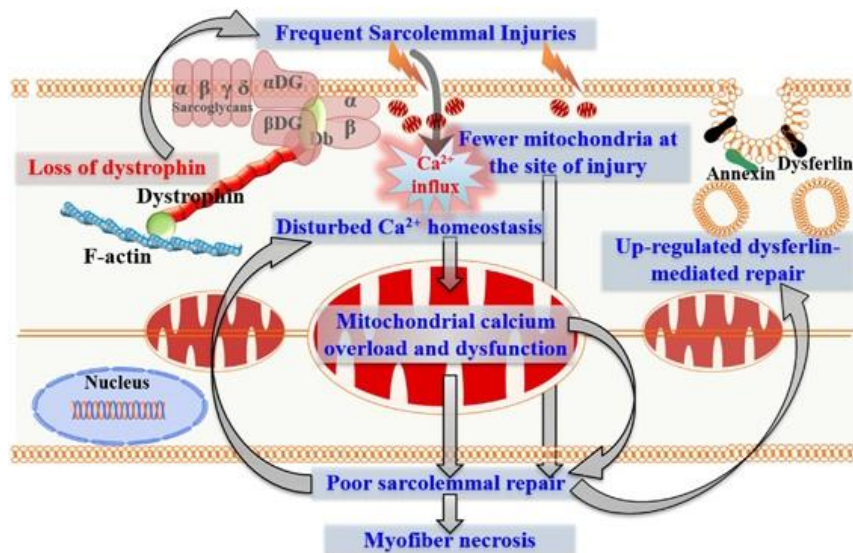


Figure 1: A model for the role of mitochondria in myofiber destruction caused by dystrophin deficiency. Dystrophin deficiency makes the myofiber sarcolemma more vulnerable to contraction-induced injury. The cellular calcium burden is increased by frequent sarcolemmal damage, partially absorbed by the mitochondria. Calcium overload causes a reduction in mitochondrial activity, which builds up at the injury site and the mitochondrial level (possibly through mitophagy). This mitochondrial disease affects the ability of dystrophin-deficient myofibers to heal themselves, resulting in a positive feedback loop that leads to necrosis and muscle degeneration in the affected muscles. A compensatory response to insufficient sarcolemmal repair, dysferlin and dysferlin-related proteins are upregulated, activating the mechanism to repair injured dystrophic sarcolemma. It was found that the increase in Dysferlin-mediated repair mechanisms slowed, but did not reverse, dystrophic sarcolemmal repair caused by decreased mitochondrial function (Vila et al., 2017).

Necroptosis mediates myofibre death in dystrophin-deficient mice

Necroptosis, a genetically regulated kind of necrosis, was recently discovered (Degterev et al., 2005). Necroptosis can be seen in dystrophin-deficient muscles in both mice and humans. RIPK1 (Receptor-interacting serine/threonine-protein kinase 1), MLKL (Mixed Lineage Kinase Domain Like Pseudokinase), and RIPK3 (Receptor-interacting serine/threonine-protein kinase 3) are all associated with necrosis *in vivo*. (He et al., 2009)(Jouan-Lanhouet et al., 2014)(Wang et al., 2014)(Trichonas et al., 2010); Fig. 2b–e shows that the expression of these three genes was higher in MDX muscle. There was significant RIPK3 immunoreactivity in MDX (Muscular Dystrophy X-linked mouse) muscle fibers, even though necrosome proteins are produced, and macrophage invasion of necrotic tissue can lead to an increase in necrosis markers (Fig. 2f,g). The immune response profile of

RIPK3 in necrotizing heart or retina is consistent with these findings (T. Zhang et al., 2016)(Huang et al., 2013).

Furthermore, necrotic conditions were linked to RIPK3-positive myofibers (Fig. 2i,j). Phosphorylation of MLKL-positive cells in the white matter of patients with multiple sclerosis or various types of liver injury is a strong indicator of a necroptotic process. Figure 2k,l shows the presence of phospho-MLKL-positive myofibers in the DMD muscle and the upregulation of *Mlk1* in TA MDX at three weeks old and MDX at nine weeks old (Morgan et al., 2018)(Ofengeim et al., 2015)(Wang et al., 2014).

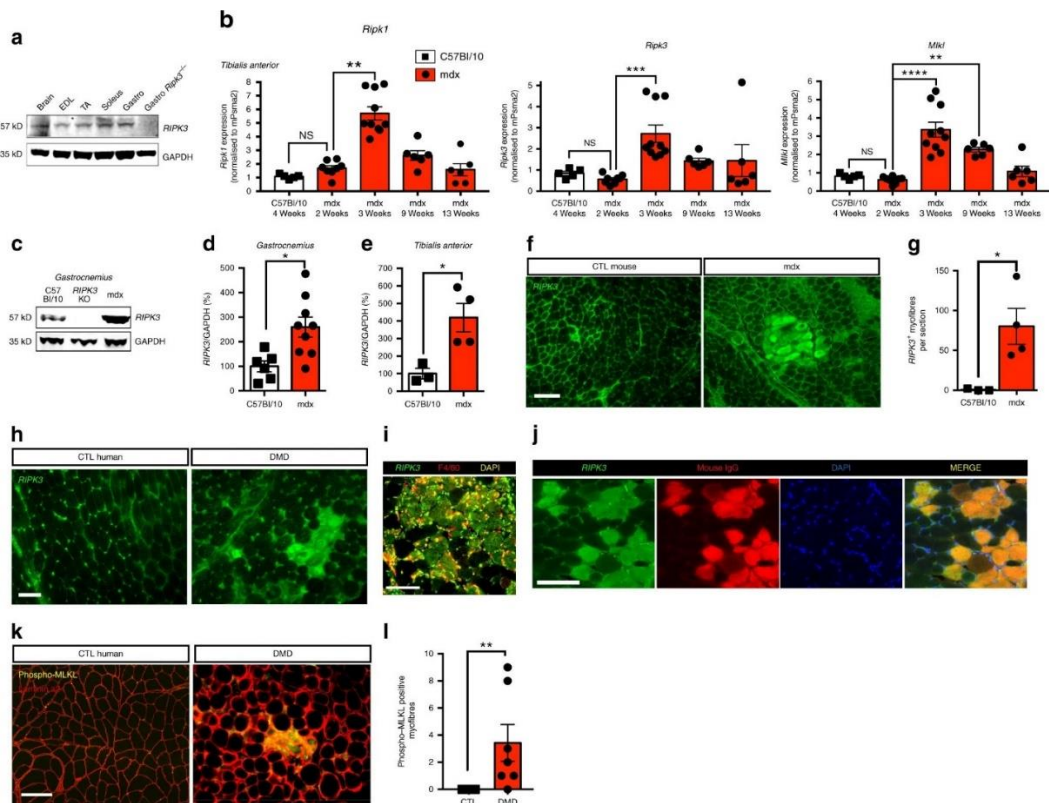


Figure 2: Induction of necroptosis in mice and humans with dystrophin deficiency. RIPK3 protein expression in C57BL/6 rat brain, extensor digitorum longus (EDL), tibialis anterior (TA), soleus, gastrocnemius muscle, and gastrocnemius muscle of RIPK3 knockout mice. As a loading control, GAPDH was used; B. Quantitative PCR was used to determine the mRNA levels of *Ripk1*, *Ripk3*, and *Mlkl* in the anterior tibialis muscle of 4-week-old C57BL/10 mice and 2, 3, 9, and 13-week-old MDX mice. Data were normalized to *Psm2* gene expression of mice for 2-, 3-, 9-, and 13-week MDX TA ($n = 5$ C57BL/10 and $n = 5, 8, 10, 6,$ and 6 for 2-, 3-, 9-, and 13 weeks MDX TA, respectively, Dunn's multiple comparison test); C. Western blot analysis of RIPK3 and GAPDH protein expression in C57BL/10, RIPK3 KO, and MDX gastrocnemius muscle extracts. RIPK3 protein expression in gastrocnemius (d) and TA (e) was normalized to GAPDH ($n = 6$ C57BL/10 and $n = 9$ MDX gastrocnemius; $n = 3$ C57BL/10 and n

= 5 MDX TA, Student's t-test); F. Antibodies to RIPK3 were used to label gastrocnemius C57BL10 mice (left) and MDX mice (right) (green); G. RIPK3-positive myofibres in the gastrocnemius of C57BL10 (n = 3) and MDX (n = 4) mice were measured. h Immunofluorescence of quadriceps biopsies from healthy non-dystrophic (human CTL) and DMD patients (green). I Immunohistochemistry with antibodies to RIPK3 (green) and mouse IgG (red), functional markers for membrane permeability in myofibres. j Immunohistochemistry utilizing antibodies against RIPK3 (green) and F4/80 (red), markers of murine macrophages. k Confocal image of a transverse cryosection of the DMD quadriceps labeled human phospho-MLKL (green), human laminin 2 (red), and DAPI antibody (blue). l Count the number of p-MLKL-positive myofibers per Fifiield (n = 5 human controls and n = 6 DMD). Data are presented as mean with standard deviation (SEM). Weighing rod, 100 μ m. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (Morgan et al., 2018).

Genes associated with Duchenne muscular dystrophy

One of the critical genes in the human genome is dystrophin, which encodes a 427 kDa protein dystrophin with 79 coding sequence exons and 2.5 Mb of DNA. Most mutations are deletions and duplications, which account for 70% to 80% of all changes. In 20 to 30 percent of cases, point mutations are discovered. Dystrophin is found in the brain and retina, in addition to striated and cardiac muscle. Although less common in the brain than in the muscles, this helps explain some of the symptoms of CNS disease (Vijay Venugopal; Steven Pavlakis, 2021). Dystrophin is a protein found in healthy muscles on the intracellular surface of the sarcolemma and along the myofibrils. This protein binds to the dystrophin-associated glycoprotein complex (DGC) on the myofiber plasma membrane to form the associated glycoprotein complex (dystroglycan, sarcoglycans, and neuronal nitric oxide synthase). Muscle contraction is dependent on dystrophin, a protein (DGC). One of the most critical functions of muscular dystrophy is to stabilize fibers during contraction by binding to the linking and anchoring proteins, F-actin and -dystroglycan (Falzarano et al., 2015).

Mutations in the Dystrophin gene: *DMD* mutations in Cohort and De novo Mutation

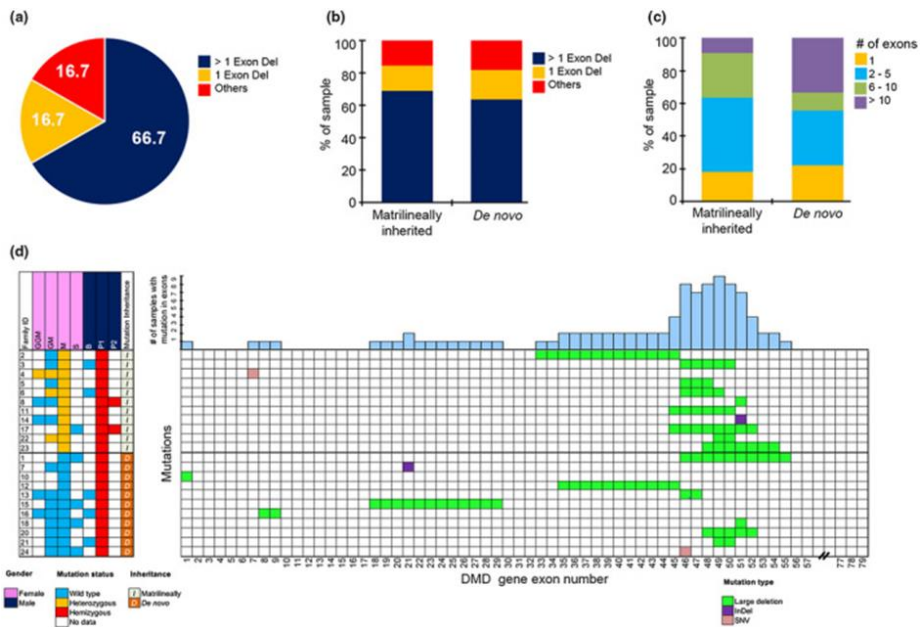


Figure 3: Overview of mutations in the cohort: (a) a measure of the frequency with which the person has an SNV, single-exon deletion, or large deletion. (b) Percentage of sporadic and maternally inherited mutations in the cohort. (c) Different exonic mutations were found in the cohort in both de novo and genetic cases, making it difficult to generalize. (d) The other DMD mutations in the cohort are shown on a heatmap (sorted by inheritance, length, type, and frequency) (Shastry et al., 2021).

For this study, they used our set of DMD probes to study the prevalence and maternal inheritance of DMD mutations in our cohort, as Sashry has done in all of his studies. Pathogenic genetic changes (SNPs, indels, large deletions) in DMD genes were found in both maternal (13 subjects, 54%) and de novo (13 subjects, 54%) subjects (11 subjects, 46 percent). In our cohort, 83.3 percent of DMD gene loss-of-function mutations were associated with large/single exon deletions. There were 16.7% single exon deletions and 66.7% multi-exon omissions in the subject samples. Each of the four subject samples contained one point mutation and one single-base indel (16.7 percent) (Shastry et al., 2021). As Sashry et al. were 11 of the 22 families in this cohort. All of the participants in the study had de novo DMD mutations. Nine families had significant exon deletions, while two others had SNVs. The DMD mutation was absent in all other maternal patients in the family.

Exons 51 in P10 (5'UTR) and P18 (5'UTR and exon 1) are the only two that are deleted, but deletions in exon 46-47, exon 8-9 in P16, exon 48-52 in P20, exon 18-29 in P15, and exons 49-50 in P21 (exon 49-50) were all significant in P1 (exon 46-55), exons 35-45 in P12 (exon 51). Exons 18-29 have been completely deleted in P15, the largest known de novo deletion. In two-thirds of DMD genes, large de novo exon deletions are distributed randomly. No pattern could be attributed to de novo mutation hotspots. There were only two de novo SNV and one single base

deletion case in the probands of the two families. Glu765AsnfsTer3 was found in exon 21 of proband P7, whereas c.6598G>T was found in exon 21 of proband P24 (p. Glu2200Ter) (Shastry et al., 2021).

Exon Deletion

Single-exon deletions in DMD were most common in exon 45 (similar to Neri et al.), as were multiple-exon deletions in exons 45 to 52. Deletions occurred in two well-established hot regions at the 5' and 3' ends of genes and were highly variable and not randomly distributed (Supplementary Fig. 1). 29 exons in the frame (exons 3, 4, 9, 15, 16, 23, 25, 26, 28, 29, 32, 33, 34, 35, 36, 37), 38, 39, 41, 42, 49, 60, 64, 71, 72, 73, 74, 75, 79) on DMD and BMD had single exon deletions. There were a total of 12 out-of-frame exon deletion sites (7, 11, 20, 59, 62, 65, 66, 67, 69, 70, 76, 77) (Fletcher et al., 2020).

CRISPR and its potential for gene editing

A virus-killing adaptive immune system found in bacteria and archaea is known as CRISPR (regularly interspaced palindromic repeat). CRISPR was first discovered by Ishino et al. In 1987, non-repetitive sequences with unknown functions were found in *E. coli*. Mojica et al. (1993) discovered repeat sequences in *Haloferax* and *Haloarcula* archaea and *M. tuberculosis* by van Soolingen et al. (1993). Although their function is still unknown, these sequences are found in more than 40% bacteria and 90% archaea. In 2002, Jansen found a gene that is always adjacent to the 'non-repeat sequences,' named Cas or the abbreviation of CRISPR associated. This case is the basis that distinguishes the types of existing CRISPR systems. There are six types of CRISPR, but because types IV to VI has just been discovered, we will only discuss a little about types I to III. Type I CRISPR uses a cascade complex; type II uses cas9, which has a significantly reduced cash protein, and type III uses CMR or Csm RAMP complexes. In 2005, Pourcel et al., Mojica et al., Bolotin et al. found that these 'non-repeat sequences' contain sequences of bacteriophage DNA. Some bacteria carrying these sequences become resistant to infection by phages because bacteriophages cannot attack the same genome with themselves. In 2007, Barrangou et al. conducted experiments on the bacterium *Streptococcus thermophilus*, proving that CRISPR is an adaptive immune system in prokaryotes (Hryhorowicz, Lipiński, Zeyland, & Słomski, 2017)(Lino, Harper, Carney, & Timlin, 2018)(Hsu, Lander, & Zhang, 2014).

Table 1
An overview of CRISPR/CAS9 usage in DMD therapies

Strategy	Target Gene Region(s)	Study Highlights	References
NHEJ reframing, HDR exon correction	<i>Dmd</i> exon 23	IHC (up to 100 percent) and WB revealed that dystrophin restoration was occurring; 17% <i>Dmd</i> Dystrophin-positive fibers in the heart and skeletal muscles were found to be 47–60	(Chengzu Long, John R. McAnally, John M.

		percent after HDR correction of these tissues;	Shelton, Alex A. Mireault, Rhonda Bassel-Duby, 2015)
NHEJ reframing, exon skipping, HDR exon knock-in	<i>DMD</i> intron 44/exon 45	Using WB and IHC, we observed the restoration of dystrophin in derived skeletal muscle cells using all of the strategies tested; CRISPR was found to be just as effective as TALEN in this regard.	(Li et al., 2015)
NHEJ reframing, single/multiple exon deletion	<i>DMD</i> exons 45–55 (for reframing each exon), introns 50 and 51 (ex51 del.), introns 44 and 55 (ex45–55 del.)	When we treated myoblasts (exon 51-deleted) in vitro, we observed dystrophin rescue by Western blot, and when we transplanted the treated myoblasts into mice, we observed dystrophin-positive fibers by immunohistochemistry, indicating that the exon 51 deletion was effective.	(David G Ousterout, Ami M Kabadi, Pratiksha I Thakore, William H Majoros, Timothy E Reddy, 2015)
NHEJ reframing, exon skipping	<i>Dmd</i> exon 51	Following the administration of systemic injections of dystrophin, improvements in muscle function were observed in the heart and skeletal muscles.	(Amoasii et al., 2017)
NHEJ reframing, exon skipping	<i>DMD</i> exon 51, introns 47, 50, 54	Dystrophin production was restored, and patient mutations were corrected in 3D-engineered cardiomyocytes, increasing contractile strength.	(Long et al., 2018)
NHEJ reframing	<i>Dmd</i> exon 23	Dystrophin can be restored, and muscle function improved using CRISPR-based CRISPR.	(Koo et al., 2018)
NHEJ reframing, exon skipping	<i>Dystrophin</i> exon 51	WB found that skeletal muscle dystrophin levels had been restored to healthy levels by 3–70% and that heart dystrophin levels had been restored by 92 percent.	(Amoasii et al., 2018)
NHEJ reframing, single/multiple exon deletion		CRISPR and TALEN approaches could be combined in one gene-editing strategy, and Cas9 and	(Maggio, Stefanucci, et al.,

	<i>DMD</i> exons 51, 53, introns 52 and 53 (ex53 del.), 43 and 54 (ex44–54 del.)	gRNA delivered either separately or together in AdV.	2016)
NHEJ reframing, single/multiple exon deletion	<i>DMD</i> exons 51, 53 (for reframing) introns 52 and 53 (ex53 del.), introns 43 and 54 (ex44–54 del.)	Using 2gRNA-SpCas9 constructs, researchers found that AdVs are just as effective at restoring dystrophin function as AdVs derived from 1gRNA-SpCas9 constructs.	(Maggio, Liu, Janssen, Chen, & Gonçalves, 2016)
NHEJ reframing, single exon skipping, HDR exon correction	<i>DMD</i> exon 51, intron 50	Five of the twenty-four pups showed HDR correction and improved dystrophic phenotypes after being injected with Cpf1 editing during the embryonic stage; this was accomplished through Cpf1 editing during the embryonic stage.	(Y. Zhang et al., 2017)
Single exon deletion	<i>Dmd</i> exon 23, introns 22 and 23 (ex23 del.)	Except for intravenous injection, all injection methods produced dystrophin-positive fibers in the skeletal and cardiac muscles, according to IHC. The following were the outcomes of this treatment: 4.6 percent and 9.6 percent in skeletal muscles, respectively. Following the injection, the skeletal and cardiac muscles increased by 1.8 percent and 3.2 percent, respectively, 12 weeks later.	(Long, Amoasii, Mireault, & Mcanally, 2016)
Single exon deletion	<i>Dmd</i> introns 22 and 23 (ex23 del.)	Exon 23 was deleted in approximately 59 percent of transcripts after intermittent intramuscular injections, resulting in approximately 8 percent of dystrophin being restored to healthy levels by WB. Injections into the system Following systemic injections, dystrophin production in the heart and skeletal muscles was restored.	(Nelson et al., 2017)
Single exon deletion	<i>Dmd</i> introns 22 and 23 (ex23 del.)	Using the Cas9 and gRNAs in different combinations on separate constructs in vitro, it was possible to increase dystrophin levels in the heart and skeletal muscles by more than 10%. The correction was	(Tabebor dbar et al., 2016)

		also possible in satellite cells after systemic Cas9 and gRNAs.	
Single exon deletion	<i>Dmd</i> introns 50 and 51 (ex51 del.)	HCAAdV delivery of CRISPR agents resulted in up to 93.3 percent exon 51 in vitro.	(Ehrke-Schulz et al., 2017)
Hybrid exon formation via internal exon deletion	<i>DMD</i> exons 50 and 54	Restoration of dystrophin in vitro by Western blot was successful; however, no evidence of this in vivo has been found.	(Ouellet et al., 2016)
Hybrid exon formation via multiple exon deletion	<i>DMD</i> exons 47 and 58	In both vitro and vivo studies, dystrophin was restored (slight rescue in the heart)	(Duchêne et al., 2018)
Multiple exon deletion	<i>Dmd</i> introns 20 and 23 (ex21–23 del.)	Following electroporation, dystrophin-associated sarcolemmal localization and muscle membrane integrity was restored to 50% of their wild-type levels, indicating that the procedure was successful, which was also restored after treatment with a calcium channel blocker (transduction).	(Xu et al., 2016)
Multiple exon deletion	<i>DMD</i> introns 44 and 55 (ex45–55 del.)	In mice, CRISPR-mediated deletion of the large exon 45–55 region of the dystrophin and dystroglycan genes restored dystrophin and dystroglycan expression, and immunohistochemistry revealed dystrophin-positive fibers in mice transplanted with hiPSC-derived skeletal muscle cells.	(Young et al., 2016)
Multiple exon deletion	<i>DMD</i> introns 44 and 55 (ex45–55 del.)	CRISPR can be used to delete exons 45–55 in vivo;	(Young, Mokhono va, Quinonez , Pyle, & Spencer, 2017)
Multiple exon deletion	<i>DMD</i> introns 2 and 7 (ex3–9 del.), introns 5 and 7 (ex6–7 del.), introns 6 and 11 (ex7–11 del.)	Ex7–11 dystrophin was the least functional, while ex3–9 dystrophin was the most functional in assessing iPSC-derived cardiomyocyte calcium cycling.	(Kyrychenko et al., 2017)
Multiple exon deletion, HDR exon correction	<i>Dmd</i> exon 53, introns 51 and 53 (ex52–53 del.)	In mice with heart failure, dystrophin expression was restored in the heart (34 percent dystrophin-positive fibers) and	(Bengtsson et al., 2017)

		skeletal muscles (10–50 percent dystrophin-positive fibers) using a dual vector approach (SpCas9 and gRNA separate).	
HDR correction	<i>Dmd</i> exon 23	CRISPR treatment and cardiotoxin injection corrected 5.4 percent of the <i>Dmd</i> mutation in mdx; however, only 0.8 percent of this mutation was corrected by CRISPR treatment alone, which resulted in significantly better hanging test performance than without the cardiotoxin co-injection.	(Lee et al., 2017)
HDR exon correction	<i>Dmd</i> exon 23	Dystrophin-positive fibres were detected by immunohistochemistry in mice that had been transplanted with corrected satellite cells, indicating that using AdVs to deliver both the Cas9 and the gRNA-HDR template resulted in increased transduction efficiency.	(Zhu et al., 2017)
Base editing to correct a nonsense mutation	<i>Dmd</i> exon 20	IHC revealed that 17.7 percent of dystrophin-positive fibers were present and that the localization of nNOS had been restored eight weeks after the treatment began. There were no off-target effects observed during this period.	(Ryu et al., 2018)
Base editing to induce exon skipping	<i>DMD</i> intron 50 5 ^o splice site	Restored dystrophin protein, low CK and miR-31 levels, and increased -dystroglycan expression after base editing demonstrated that base editing was 100% effective.	(Yuan et al., 2018)
Duplicated exon removal	<i>DMD</i> exon 2, intron 2	WB and IHC can partially rescue dystrophin after deleting an extra exon by a single gRNA.	(Lattanzi et al., 2017)
Duplicated exons removal	<i>DMD</i> intron 27	Following treatment, it was discovered that 4.42 percent of full-length dystrophin was produced, accompanied by a restoration of -dystroglycan.	(Wojtal et al., 2016)
Utrophin upregulation	<i>UTRN</i> A/B promoter	It was possible to achieve a restored -dystroglycan expression level with as little as a 1.7-fold increase in utrophin	(Wojtal et al., 2016)

	expression, which was detected by Western blot as early as 24 hours after the start of the experiment.	
--	--	--

Lim, Yoon, and Yokota stated that “several abbreviations for non-homologous end joining (NHEJ) and homology-directed repair (HDR) are used, including exon, exons, NSG, NOD SCID IL2R gamma, and hiPSCs (HDR). The terms lentivirus, AAV, adenovirus, and HCAv (high-capability adenoviral vector) are all acronyms for high-capability adenoviral vectors; PEI stands for polyethyleneimine; i.m. stands for intramuscular; i.p. stands for intraperitoneal; i.v. stands for intravenous; Western blot, IHC, and HCAv are all acronyms for high- The transcription activator-like effector nuclease (TALEN) is a nuclease that functions as a transcription activator (nNOS).”(Lim, Yoon, & Yokota, 2018)

CRISPR mechanism in gene editing

Gene-edited animals are used for basic research, disease modeling, preclinical drug testing, and transplantation because CRISPR/Cas9 can cause double-stranded DNA breaks (DSBs) at particular genomic sites that can be repaired by one of two mechanisms. Resources should be improved (Doetschman & Georgieva, 2017). For exogenous DNA, the role of CRISPR-Cas is broken down into three stages: Adaptation (the process of acquiring a spacer), Affirmation (the process of CRISPR RNA [crRNA]), and Interference (the destruction and cutting of invading DNA or RNA).The invader was gone in a matter of minutes. After a protospacer is isolated from the invading DNA (such as phage or plasmid), the Cas1-Cas2 protein complex can be inserted into the 5' end of a CRISPR array and integrated into the host chromosome during the adaptation phase. This inserted fragment represents this new spacer unit. The Cas1-Cas2 protein complex comprises two Cas1 units and a Cas2 unit. The production of crRNA is the second step. There has been a lot of transcription of the CRISPR array (repeat and spacer sequences) so far, which has been processed by cleavage and splitting into short RNAs (pre-rRNAs) (crRNAs or mature crRNAs). In the interference phase, the crRNA–Cas complex and crRNA–Cas9 can cleave invader/target DNA or RNA (Amitai & Sorek, 2016).

CRISPR/Cas9 potential in Duchenne muscular dystrophy : A promising role of CRISPR/cas9 in DMD, MLKL, and RIPK3 gene

By recruiting and phosphorylating the executioner MLKL, which is released by necroptosis-induced cytoplasmic membrane rupture and intracellular Leakage-induced signaling, RIPK3 is the master regulator of this mode of cell death. The content of cells that are dying is wiped out. RIPK3 and MLKL, according to current knowledge, are essential mechanisms for all necrotic cell death responses. In necroptosis-sensitive cell lines, we used CRISPR/Cas9 technology to demonstrate that Ripk3 and Mille knockout and Ripk3/Mille double knockout reduced necroptosis susceptibility to death to an undetectable degree in both cases. A drug target for necroptosis-mediated pathology was high-dose tumor necrosis factor (TNF), which was demonstrated in vivo using Ripk3- or Mlk1-deficient mice (Moerke, Bleibaum, Kunzendorf, & Krautwald, 2019).

The reason using CRISPR/Cas9 As an Effective therapy for Duchenne muscular dystrophy

Restoring dystrophin expression in the skeletal and cardiac muscle through CRISPR gene editing opens up new treatment avenues for the disease. This method has been tested in rodents, large animals, and human cells, proving its viability (Olson, 2021). Many CRISPR-mediated DMD gene-editing studies are discussed here, both in vitro and in vivo. The CRISPR system now includes base or primary editors, allowing for even finer control over gene editing. In recent years, B. delivery, off-target mutagenesis, and long-term dystrophin maintenance have improved (Erkut & Yokota, 2022).

Table 2
Comparison corticosteroid Vs CRISPR/Cas9 as therapeutic method in Duchenne muscular dystrophy

Indicator	Corticosteroids	CRISPR/Cas9
Definition	Corticosteroid is the gold standard therapy that can delayed progression of DMD (Falzarano et al., 2015).	CRISPR is one of genome engineering that can edit sequence specifically in the genome (Lim et al., 2018).
Mechanism	There are numerous benefits to corticosteroid therapy, including improving muscle strength by increasing walking time and keeping the upper extremities functional, helping to reduce the progression of cardiomyopathy, and delaying the need for invasive ventilation, with some patients surviving for more than 30 years (Werneck et al., 2019).	As a result of its ability to permanently bypass exons, CRISPR/Cas9 technology has gained interest as a treatment option for Duchenne muscular dystrophy (DMD) (Lim et al., 2018). It has been estimated that ~60% of DMD patients could benefit from gene editing strategies that skip the out-of-frame exons within these hotspots, and up to 80% of the patient population could potentially benefit if additional less common mutations were corrected (Min, Bassel-Duby, & Olson, 2019).
Efficacy	The efficacy of prednisone and deflazacort (corticosteroids) in short-term treatment studies (six months to two years) was comparable, with clinically	When CRISPR/Cas9 therapy induces p53-dependent DNA damage response, this barrier to efficacy becomes increasingly apparent. In

	non-serious side effects improving muscle strength and function (Falzarano et al., 2015).	vitro gene repair efficiency is reduced when CRISPR/Cas9-generated double-stranded DNA breaks activate p53 (Lim et al., 2018).
Benefit	Nonrandomized studies found that taking prednisone or desflurane for more than two years had significant positive effects on walking ability and cardiac function, delayed the onset of scoliosis and respiratory dysfunction and improved overall quality of life (Falzarano et al., 2015).	In the long run, CRISPR/Cas9 will save patients with DMD money by permanently correcting genetic defects (Lim et al., 2018).
Risk	Adrenal suppression and bone mineral loss can occur due to prolonged corticosteroid use. Growth retardation and puberty can also be a result of long-term corticosteroid use (Werneck et al., 2019).	Due to the lack of control over the editing process, CRISPR/Cas9-induced mutations in treated cells pose a significant danger (Lim et al., 2018). Unintended genome editing results were found in 16 percent of the human embryo cells studied and ranged in size from 4 to 20 kb (Alanis-Lobato et al., 2021).

Human illnesses such as cancer, genetic, immunological, neurological problems, and viral infections are increasingly being treated with CRISPR/Cas9-based techniques. These CRISPR/Cas9 techniques may be utilized not just for treatment but also for disease modeling, leading to a better knowledge of the processes of numerous viral and genetic disorders. Furthermore, the CRISPR/Cas9 technology may be employed as programmable antibiotics to kill specific bacterium sequences, bypassing multidrug resistance. Additionally, CRISPR/Cas9-based gene drive has the potential to reduce the transmission of vector-borne illnesses (Khan et al., 2018).

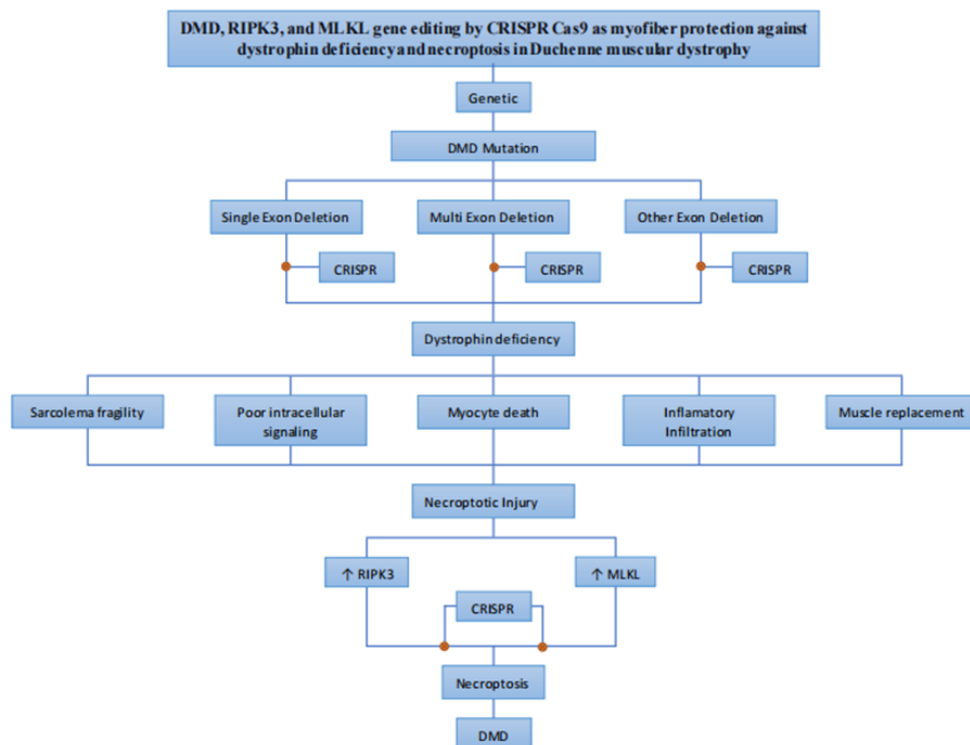


Figure 4. The framework of the deletion DMD, RIPK3, and MLKL in preventing the myofiber death and necroptosis

Acknowledgments

Dr.dr. Fitri Handajani M.Kes (University of Hang Tuah) and Dr. Firman Suryadi Rahman (Universitas Airlangga) were extremely helpful in providing answers and criticism on the article. We also appreciate Arga Setyo Adji (University of Hang Tuah), Vira Aulia Kusuma Wardani (University of Hang Tuah), Eilien Levina Santoso (University of Hang Tuah), and Fadhillah Rachmawati Sunarto (University of Hang Tuah), for their insightful conversations. Hang Tuah University's Faculty of Medicine funded the study.

References

- Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, 5(1), i-v. <https://doi.org/10.53730/ijhs.v5n1.2864>
- Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). The COVID-19 pandemic. *International Journal of Health Sciences*, 5(2), vi-ix. <https://doi.org/10.53730/ijhs.v5n2.2937>
- Gustiani, R., Hakimi, M., & Suryaningsih, E. K. (2022). The implementation of referral system in postpartum hemorrhage cases by midwife. *International Journal of Health & Medical Sciences*, 5(3). <https://doi.org/10.21744/ijhms.v5n3.1917>

- Aartsma-Rus, A., Van Deutekom, J. C. T., Fokkema, I. F., Van Ommen, G. J. B., & Den Dunnen, J. T. (2006). Entries in the Leiden Duchenne muscular dystrophy mutation database: An overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle and Nerve*, 34(2), 135–144. <https://doi.org/10.1002/mus.20586>
- Alanis-Lobato, G., Zohren, J., McCarthy, A., Fogarty, N. M. E., Kubikova, N., Hardman, E., ... Niakan, K. K. (2021). Frequent loss of heterozygosity in CRISPR-Cas9–edited early human embryos. *Proceedings of the National Academy of Sciences*, 118(22). <https://doi.org/10.1073/pnas.2004832117>
- Amitai, G., & Sorek, R. (2016). CRISPR–Cas adaptation: insights into the mechanism of action. *Nature Publishing Group*, 1–10. <https://doi.org/10.1038/nrmicro.2015.14>
- Amoasii, L., Hildyard, J. C. W., Li, H., Sanchez-Ortiz, E., Mireault, A., Caballero, D., ... Olson, E. N. (2018). Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy. *Science*, 362(6410), 86–91. <https://doi.org/10.1126/science.aau1549>
- Amoasii, L., Long, C., Li, H., Mireault, A. A., Shelton, J. M., Sanchez-Ortiz, E., ... Olson, E. N. (2017). Single-cut genome editing restores dystrophin expression in a new mouse model of muscular dystrophy. *Science Translational Medicine*, 9(418). <https://doi.org/10.1126/scitranslmed.aan8081>
- Bello, L., & Pegoraro, E. (2019). The “usual suspects”: Genes for inflammation, fibrosis, regeneration, and muscle strength modify duchenne muscular dystrophy. *Journal of Clinical Medicine*, 8(5), 1–23. <https://doi.org/10.3390/jcm8050649>
- Bengtsson, N. E., Hall, J. K., Odom, G. L., Phelps, M. P., Andrus, C. R., Hawkins, R. D., ... Chamberlain, J. S. (2017). Muscle-specific CRISPR/Cas9 dystrophin gene editing ameliorates pathophysiology in a mouse model for Duchenne muscular dystrophy. *Nature Communications*, 8, 1–9. <https://doi.org/10.1038/ncomms14454>
- Birnkrant, D. J., Bushby, K., Bann, C. M., Apkon, S. D., Blackwell, A., Brumbaugh, D., ... Weber, D. R. (2018, March). Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. *The Lancet Neurology*, Vol. 17, pp. 251–267. [https://doi.org/10.1016/S1474-4422\(18\)30024-3](https://doi.org/10.1016/S1474-4422(18)30024-3)
- Chang, N. C., Chevalier, F. P., & Rudnicki, M. A. (2016). Satellite Cells in Muscular Dystrophy - Lost in Polarity. *Trends in Molecular Medicine*, 22(6), 479–496. <https://doi.org/10.1016/j.molmed.2016.04.002>
- Chengzu Long, John R. McAnally, John M. Shelton, Alex A. Mireault, Rhonda Bassel-Duby, E. N. O. (2015). Prevention of muscular dystrophy in mice by CRISPR/Cas9–mediated editing of germline DNA. 345(6201), 1184–1188. <https://doi.org/10.1126/science.1254445.Prevention>
- Cornelis, E., & Gessal, J. (2021). Duchene Muscular Disorder. *Jurnal Medik Dan Rehabilitasi*, 3(1), 1–10. Retrieved from <https://ejournal.unsrat.ac.id/index.php/jmr/article/view/32925>
- David G Ousterout , Ami M Kabadi , Pratiksha I Thakore , William H Majoros , Timothy E Reddy, C. A. G. (2015). Multiplex CRISPR/Cas9-Based Genome Editing for Correction of Dystrophin Mutations that Cause Duchenne Muscular Dystrophy David. HHS Public Access. <https://doi.org/10.1038/ncomms7244.Multiplex>

- De Los Angeles Beytía, M., Vry, J., & Kirschner, J. (2012). Drug treatment of Duchenne muscular dystrophy: Available evidence and perspectives. *Acta Myologica*, 31(MAY), 4–8.
- Degtarev, A., Huang, Z., Boyce, M., Li, Y., Jagtap, P., Mizushima, N., ... Yuan, J. (2005). Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nature Chemical Biology*, 1(2), 112–119. <https://doi.org/10.1038/nchembio711>
- Doetschman, T., & Georgieva, T. (2017). Gene Editing With CRISPR/Cas9 RNA-Directed Nuclease. 876–895. <https://doi.org/10.1161/CIRCRESAHA.116.309727>
- Duchêne, B. L., Cherif, K., Iyombe-Engembe, J. P., Guyon, A., Rousseau, J., Ouellet, D. L., ... Tremblay, J. P. (2018). CRISPR-Induced Deletion with SaCas9 Restores Dystrophin Expression in Dystrophic Models In Vitro and In Vivo. *Molecular Therapy*, 26(11), 2604–2616. <https://doi.org/10.1016/j.ymthe.2018.08.010>
- Ehrke-Schulz, E., Schiwon, M., Leitner, T., Dávid, S., Bergmann, T., Liu, J., & Ehrhardt, A. (2017). CRISPR/Cas9 delivery with one single adenoviral vector devoid of all viral genes. *Scientific Reports*, 7(1), 1–11. <https://doi.org/10.1038/s41598-017-17180-w>
- Erkut, E., & Yokota, T. (2022). CRISPR Therapeutics for Duchenne Muscular Dystrophy. *International Journal of Molecular Sciences*, 23(3), 1832. <https://doi.org/10.3390/ijms23031832>
- Falzarano, M. S., Scotton, C., Passarelli, C., & Ferlini, A. (2015). Duchenne muscular dystrophy: From diagnosis to therapy. *Molecules*, 20(10), 18168–18184. <https://doi.org/10.3390/molecules201018168>
- Fayssoil, A., Nardi, O., Orlikowski, D., & Annane, D. (2010). Cardiomyopathy in Duchenne muscular dystrophy: pathogenesis and therapeutics. *Heart Failure Reviews*, 15(1), 103–107. <https://doi.org/10.1007/s10741-009-9156-8>
- Fletcher, S., Neri, M., Rossi, R., TrabANELLI, C., Mauro, A., Selvatici, R., ... Tessa, A. (2020). The Genetic Landscape of Dystrophin Mutations in Italy: A Nationwide Study. 11(March), 1–15. <https://doi.org/10.3389/fgene.2020.00131>
- Fong, P., Turner, P. R., Denetclaw, W. F., & Steinhardt, R. A. (1990). Increased Activity of Calcium Leak Channels in Myotubes of Duchenne Human and mdx Mouse Origin. *Science*, 250(4981), 673–676. <https://doi.org/10.1126/science.2173137>
- Gao, Q. Q., & McNally, E. M. (2015). The dystrophin complex: Structure, function, and implications for therapy. *Comprehensive Physiology*, 5(3), 1223–1239. <https://doi.org/10.1002/cphy.c140048>
- Gupta, R., Sinharoy, S., Acharya, K., & Ghosh, D. (2019). CRISPR-Cas9 system: A new-fangled dawn in gene editing. *Life Sciences*, 116636. <https://doi.org/10.1016/j.lfs.2019.116636>
- He, S., Wang, L., Miao, L., Wang, T., Du, F., Zhao, L., & Wang, X. (2009). Receptor Interacting Protein Kinase-3 Determines Cellular Necrotic Response to TNF- α . *Cell*, 137(6), 1100–1111. <https://doi.org/10.1016/j.cell.2009.05.021>
- Hryhorowicz, M., Lipiński, D., Zeyland, J., & Słomski, R. (2017). CRISPR/Cas9 Immune System as a Tool for Genome Engineering. *Archivum Immunologiae et Therapiae Experimentalis*, 65(3), 233–240. <https://doi.org/10.1007/s00005-016-0427-5>

- Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. *Cell*, 157(6), 1262–1278. <https://doi.org/10.1016/j.cell.2014.05.010>
- Huang, J.-F., Shang, L., Zhang, M.-Q., Wang, H., Chen, D., Tong, J.-B., ... Xiong, K. (2013). Differential neuronal expression of receptor interacting protein 3 in rat retina: involvement in ischemic stress response. *BMC Neuroscience*, 14(1), 16. <https://doi.org/10.1186/1471-2202-14-16>
- Janik, E., Niemcewicz, M., Ceremuga, M., & Krzowski, L. (n.d.). Various Aspects of a Gene Editing System — CRISPR – Cas9.
- Jiang, C., Meng, L., Yang, B., & Luo, X. (n.d.). Application of CRISPR/Cas9 gene editing technique in the study of cancer treatment. <https://doi.org/10.1111/cge.13589>
- Jouan-Lanhouet, S., Riquet, F., Duprez, L., Vanden Berghe, T., Takahashi, N., & Vandenabeele, P. (2014). Necroptosis, in vivo detection in experimental disease models. *Seminars in Cell & Developmental Biology*, 35, 2–13. <https://doi.org/10.1016/j.semcdb.2014.08.010>
- Khan, S., Mahmood, M. S., Rahman, S. U., Zafar, H., Habibullah, S., Khan, Z., & Ahmad, A. (2018). CRISPR/Cas9: The Jedi against the dark empire of diseases. *Journal of Biomedical Science*, 25(1), 1–18. <https://doi.org/10.1186/s12929-018-0425-5>
- Koo, T., Lu-Nguyen, N. B., Malerba, A., Kim, E., Kim, D., Cappellari, O., ... Kim, J. S. (2018). Functional Rescue of Dystrophin Deficiency in Mice Caused by Frameshift Mutations Using *Campylobacter jejuni* Cas9. *Molecular Therapy*, 26(6), 1529–1538. <https://doi.org/10.1016/j.ymthe.2018.03.018>
- Kyrychenko, V., Kyrychenko, S., Tiburcy, M., Shelton, J. M., Long, C., Schneider, J. W., ... Olson, E. N. (2017). Functional correction of dystrophin actin binding domain mutations by genome editing. *JCI Insight*, 2(18), 1–16. <https://doi.org/10.1172/JCI.INSIGHT.95918>
- Lattanzi, A., Moiani, A., Izmiryan, A., Martin, S., Mavilio, F., Bovolenta, M., ... Bernardi, F. (2017). Correction of the Exon 2 Duplication in DMD Myoblasts by a Single CRISPR/Cas9 System. *Molecular Therapy - Nucleic Acids*, 7(June), 11–19. <https://doi.org/10.1016/j.omtn.2017.02.004>
- Lee, K., Conboy, M., Park, H. M., Jiang, F., Kim, H. J., Dewitt, M. A., ... Murthy, N. (2017). Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA in vivo induces homology-directed DNA repair. *Nature Biomedical Engineering*, 1(11), 889–901. <https://doi.org/10.1038/s41551-017-0137-2>
- Li, H. L., Fujimoto, N., Sasakawa, N., Shirai, S., Ohkame, T., Sakuma, T., ... Hotta, A. (2015). Precise Correction of the Dystrophin Gene in Duchenne Muscular Dystrophy Patient Induced Pluripotent Stem Cells by TALEN and CRISPR-Cas9. *Stem Cell Reports*, 4(1), 143–154. <https://doi.org/10.1016/j.stemcr.2014.10.013>
- Lim, K. R. Q., Yoon, C., & Yokota, T. (2018). Applications of CRISPR/Cas9 for the treatment of duchenne muscular dystrophy. *Journal of Personalized Medicine*, 8(4). <https://doi.org/10.3390/jpm8040038>
- Lino, C. A., Harper, J. C., Carney, J. P., & Timlin, J. A. (2018). Delivering crispr: A review of the challenges and approaches. *Drug Delivery*, 25(1), 1234–1257. <https://doi.org/10.1080/10717544.2018.1474964>
- Long, C., Amoasii, L., Mireault, A. A., & Mcanally, J. R. (2016). Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular

- dystrophy. 351(6271), 400–403.
<https://doi.org/10.1126/science.aad5725>. Postnatal
- Long, C., Li, H., Tiburcy, M., Rodriguez-Caycedo, C., Kyrychenko, V., Zhou, H., ... Olson, E. N. (2018). Correction of diverse muscular dystrophy mutations in human engineered heart muscle by single-site genome editing. *Science Advances*, 4(1), 1–11. <https://doi.org/10.1126/sciadv.aap9004>
- Maggio, I., Liu, J., Janssen, J. M., Chen, X., & Gonçalves, M. A. F. V. (2016). Adenoviral vectors encoding CRISPR / Cas9 multiplexes rescue dystrophin synthesis in unselected populations of DMD muscle cells. *Nature Publishing Group*, (June), 1–12. <https://doi.org/10.1038/srep37051>
- Maggio, I., Stefanucci, L., Janssen, J. M., Liu, J., Chen, X., Mouly, V., & Gonc, M. A. F. V. (2016). Selection-free gene repair after adenoviral vector transduction of designer nucleases: rescue of dystrophin synthesis in DMD muscle cell populations. 44(3), 1449–1470. <https://doi.org/10.1093/nar/gkv1540>
- Mah, J. K. (2016). Current and emerging treatment strategies for Duchenne muscular dystrophy. *Current Treatment Options in Neurology*, 12, 1795–1807. <https://doi.org/10.1007/s11940-018-0513-6>
- McCarter, G. C., & Steinhardt, R. A. (2000). Increased Activity of Calcium Leak Channels Caused by Proteolysis Near Sarcolemmal Ruptures. *The Journal of Membrane Biology*, 176(2), 169–174. <https://doi.org/10.1007/s00232001086>
- Milanti Dewi, M., Putro Widodo, D., Amardiyanto, R., Sinaga, N., & Hidayah, N. (2018). Prevalensi, Spektrum Klinis dan Gambaran Neurofisiologi Kasus Neuromuskular. 20(4).
- Min, Y.-L., Bassel-Duby, R., & Olson, E. N. (2019). CRISPR Correction of Duchenne Muscular Dystrophy. *Annual Review of Medicine*, 70(1), 239–255. <https://doi.org/10.1146/annurev-med-081117-010451>
- Moat, S. J., Bradley, D. M., Salmon, R., Clarke, A., & Hartley, L. (2013). Newborn bloodspot screening for Duchenne Muscular Dystrophy: 21 years experience in Wales (UK). *European Journal of Human Genetics*, 21(10), 1049–1053. <https://doi.org/10.1038/ejhg.2012.301>
- Moerke, C., Bleibaum, F., Kunzendorf, U., & Krautwald, S. (2019). Combined Knockout of RIPK3 and MLKL Reveals Unexpected Outcome in Tissue Injury and Inflammation. *Frontiers in Cell and Developmental Biology*, 7(February), 1–6. <https://doi.org/10.3389/fcell.2019.00019>
- Morgan, J. E., Prola, A., Mariot, V., Pini, V., Meng, J., Hourde, C., ... Bencze, M. (2018). Necroptosis mediates myofibre death in dystrophin-deficient mice. *Nature Communications*, 9(1). <https://doi.org/10.1038/s41467-018-06057-9>
- Nelson, C. E., Hakim, C. H., Ousterout, D. G., Thakore, P. I., Moreb, E. A., Rivera, R. M. C., ... Ann, F. (2017). In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. 351(6271), 403–407. <https://doi.org/10.1126/science.aad5143>. In
- Ng, R., Metzger, J. M., Claflin, D. R., & Faulkner, J. A. (2008). Poloxamer 188 reduces the contraction-induced force decline in lumbrical muscles from mdx mice. *American Journal of Physiology-Cell Physiology*, 295(1), C146–C150. <https://doi.org/10.1152/ajpcell.00017.2008>
- Ofengeim, D., Ito, Y., Najafov, A., Zhang, Y., Shan, B., DeWitt, J. P., ... Yuan, J. (2015). Activation of Necroptosis in Multiple Sclerosis. *Cell Reports*, 10(11), 1836–1849. <https://doi.org/10.1016/j.celrep.2015.02.051>

- Olson, E. N. (2021). Toward the correction of muscular dystrophy by gene editing. *Proceedings of the National Academy of Sciences of the United States of America*, 118(22). <https://doi.org/10.1073/PNAS.2004840117>
- Ouellet, D. L., Barbeau, X., Rousseau, J., Chapdelaine, P., Lagüe, P., & Tremblay, J. P. (2016). Efficient Restoration of the Dystrophin Gene Reading Frame and Protein Structure in DMD Myoblasts Using the CinDel Method. (January), 1–12. <https://doi.org/10.1038/mtna.2015.58>
- Paquin, R. S., Fischer, R., Mansfield, C., Mange, B., Beaverson, K., Ganot, A., ... Peay, H. L. (2019). Priorities when deciding on participation in early-phase gene therapy trials for Duchenne muscular dystrophy: A best-worst scaling experiment in caregivers and adult patients. *Orphanet Journal of Rare Diseases*, 14(1), 1–9. <https://doi.org/10.1186/s13023-019-1069-6>
- Porter, G. A., Dmytrenko, G. M., Winkelmann, J. C., & Bloch, R. J. (1992). Dystrophin colocalizes with β -spectrin in distinct subsarcolemmal domains in mammalian skeletal muscle. *Journal of Cell Biology*, 117(5), 997–1005. <https://doi.org/10.1083/jcb.117.5.997>
- Romitti, P. A., Zhu, Y., Puzhankara, S., James, K. A., Nabukera, S. K., Zamba, G. K. D., ... Bolen, J. (2015). Prevalence of Duchenne and Becker Muscular Dystrophies in the United. 135(3).
- Ryder, S., Leadley, R. M., Armstrong, N., Westwood, M., De Kock, S., Butt, T., ... Kleijnen, J. (2017). The burden, epidemiology, costs and treatment for Duchenne muscular dystrophy: An evidence review. *Orphanet Journal of Rare Diseases*, 12(1), 1–21. <https://doi.org/10.1186/s13023-017-0631-3>
- Ryu, S. M., Koo, T., Kim, K., Lim, K., Baek, G., Kim, S. T., ... Kim, J. S. (2018). Adenine base editing in mouse embryos and an adult mouse model of Duchenne muscular dystrophy. *Nature Biotechnology*, 36(6), 536–539. <https://doi.org/10.1038/nbt.4148>
- Shastri, A., Aravind, S., Sunil, M., Ramesh, K., Ashley, B., Vedam, N. T., ... Phalke, S. (2021). Matrilineal analysis of mutations in the DMD gene in a multigenerational South Indian cohort using DMD gene panel sequencing. (January), 1–12. <https://doi.org/10.1002/mgg3.1633>
- Sinha, R., Sarkar, S., Khaitan, T., & Dutta, S. (2017). Duchenne muscular dystrophy: Case report and review. *Journal of Family Medicine and Primary Care*, 6(3), 654. <https://doi.org/10.4103/2249-4863.222015>
- Sun, C., Serra, C., Lee, G., & Wagner, K. R. (2020). Stem cell-based therapies for Duchenne muscular dystrophy. *Experimental Neurology*, 323(Dmd), 1–28. <https://doi.org/10.1016/j.expneurol.2019.113086>
- Tabebordbar, M., Zhu, K., Cheng, J. K. W., Chew, W. L., Widrick, J. J., Yan, W. X., ... Amy, J. (2016). In vivo gene editing in dystrophic mouse muscle and muscle stem cells. 351(6271), 407–411. <https://doi.org/10.1126/science.aad5177>
- Takeda, S., Clemens, P. R., & Hoffman, E. P. (2021). Exon-Skipping in Duchenne Muscular Dystrophy. *Journal of Neuromuscular Diseases*, 8(s2), S343–S358. <https://doi.org/10.3233/JND-210682>
- Tomar, S., Moorthy, V., Sethi, R., Chai, J., Low, P. S., Hong, S. T. K., & Lai, P. S. (2019). Mutational spectrum of dystrophinopathies in Singapore: Insights for genetic diagnosis and precision therapy. *American Journal of Medical Genetics, Part C: Seminars in Medical Genetics*, 181(2), 230–244. <https://doi.org/10.1002/ajmg.c.31704>

- Trichonas, G., Murakami, Y., Thanos, A., Morizane, Y., Kayama, M., Debouck, C. M., ... Vavvas, D. G. (2010). Receptor interacting protein kinases mediate retinal detachment-induced photoreceptor necrosis and compensate for inhibition of apoptosis. *Proceedings of the National Academy of Sciences*, 107(50), 21695–21700. <https://doi.org/10.1073/pnas.1009179107>
- Vijay Venugopal; Steven Pavlakis. (2021). *Duchenne Muscular Dystrophy*. StatPearls Publishing.
- Vila, M. C., Rayavarapu, S., Hogarth, M. W., Van Der Meulen, J. H., Horn, A., Defour, A., ... Jaiswal, J. K. (2017). Mitochondria mediate cell membrane repair and contribute to Duchenne muscular dystrophy. *Cell Death and Differentiation*, 24(2), 330–342. <https://doi.org/10.1038/cdd.2016.127>
- Wang, H., Sun, L., Su, L., Rizo, J., Liu, L., Wang, L.-F., ... Wang, X. (2014). Mixed Lineage Kinase Domain-like Protein MLKL Causes Necrotic Membrane Disruption upon Phosphorylation by RIP3. *Molecular Cell*, 54(1), 133–146. <https://doi.org/10.1016/j.molcel.2014.03.003>
- Werneck, L. C., Lorenzoni, P. J., Ducci, R. D. P., Fustes, O. H., Kay, C. S. K., & Scola, R. H. (2019). Duchenne muscular dystrophy: An historical treatment review. *Arquivos de Neuro-Psiquiatria*, 77(8), 579–589. <https://doi.org/10.1590/0004-282X20190088>
- Wojtal, D., Kemaladewi, D. U., Malam, Z., Abdullah, S., Wong, T. W. Y., Hyatt, E., ... Ivakine, E. A. (2016). Spell Checking Nature : Versatility of CRISPR / Cas9 for Developing Treatments for Inherited Disorders. *The American Journal of Human Genetics*, 98(1), 90–101. <https://doi.org/10.1016/j.ajhg.2015.11.012>
- Xu, L., Park, K. H., Zhao, L., Xu, J., Refaey, M. El, Gao, Y., ... Han, R. (2016). CRISPR-mediated Genome Editing Restores Dystrophin Expression and Function in mdx Mice. 24(3), 564–569. <https://doi.org/10.1038/mt.2015.192>
- Yao, S., Chen, Z., Yu, Y., Zhang, N., Jiang, H., Zhang, G., ... Zhang, B. (2021). Current Pharmacological Strategies for Duchenne Muscular Dystrophy. *Frontiers in Cell and Developmental Biology*, Vol. 9. <https://doi.org/10.3389/fcell.2021.689533>
- Yeung, E. W., Whitehead, N. P., Suchyna, T. M., Gottlieb, P. A., Sachs, F., & Allen, D. G. (2005). Effects of stretch-activated channel blockers on [Ca²⁺]_i and muscle damage in the mdx mouse. *The Journal of Physiology*, 562(2), 367–380. <https://doi.org/10.1113/jphysiol.2004.075275>
- Young, C. S., Hicks, M. R., Ermolova, N. V., Nakano, H., Jan, M., Younesi, S., ... Pyle, A. D. (2016). A Single CRISPR-Cas9 Deletion Strategy that Targets the Majority of DMD Patients Restores Dystrophin Function in hiPSC-Derived Muscle Cells. *Cell Stem Cell*, 18(4), 533–540. <https://doi.org/10.1016/j.stem.2016.01.021>
- Young, C. S., Mokhonova, E., Quinonez, M., Pyle, A. D., & Spencer, M. J. (2017). Creation of a Novel Humanized Dystrophic Mouse Model of Duchenne Muscular Dystrophy and Application of a CRISPR/Cas9 Gene Editing Therapy. *Journal of Neuromuscular Diseases*, 4(2), 139–145. <https://doi.org/10.3233/JND-170218>
- Yuan, J., Ma, Y., Huang, T., Chen, Y., Peng, Y., Li, B., ... Chang, X. (2018). Genetic Modulation of RNA Splicing with a CRISPR-Guided Cytidine Deaminase. *Molecular Cell*, 72(2), 380–394.e7. <https://doi.org/10.1016/j.molcel.2018.09.002>
- Zhang, T., Zhang, Y., Cui, M., Jin, L., Wang, Y., Lv, F., ... Xiao, R.-P. (2016). CaMKII is a RIP3 substrate mediating ischemia- and oxidative stress-induced

- myocardial necroptosis. *Nature Medicine*, 22(2), 175–182.
<https://doi.org/10.1038/nm.4017>
- Zhang, Y., Long, C., Li, H., McAnally, J. R., Baskin, K. K., Shelton, J. M., ... Olson, E. N. (2017). CRISPR-Cpf1 correction of muscular dystrophy mutations in human cardiomyocytes and mice. *Science Advances*, 3(4), 1–10.
<https://doi.org/10.1126/sciadv.1602814>
- Zhu, P., Wu, F., Mosenson, J., Zhang, H., He, T. C., & Wu, W. S. (2017). CRISPR/Cas9-Mediated Genome Editing Corrects Dystrophin Mutation in Skeletal Muscle Stem Cells in a Mouse Model of Muscle Dystrophy. *Molecular Therapy - Nucleic Acids*, 7(June), 31–41.
<https://doi.org/10.1016/j.omtn.2017.02.007>