flanked by AAV integration-enhancing elements was tested for targeted integration.[12]

Gene correction is a process whereby sequence alterations in genes can be corrected by homologous recombination-mediated gene conversion between the recipient target locus and a donor construct encoding the correct sequence.[13] The introduction of a corrective sequence together with a site-specific nuclease to induce a double-stranded break (DSB) at sites responsible for monogenic disorders would activate gene correction. Pairs of designated zinc-finger protein with tandem DNA binding sites fused to the cleavage domain of the FokI protein were introduced into model systems or cell lines and produced corrections in 10–30% of cases tested.[14]

## 2.2. *Modification of the dystrophin Gene and Promoter*

Due to the large deletion in its genome, the gutted adenovirus vector can package 14-kb of full-length *dystrophin* cDNA. Multiple proximal muscles of seven-day-old utrophin/dystrophin double knockout mice (*dko* mice), which typically show symptoms similar to human DMD, were effectively transduced with the gutted adenovirus bearing full-length murine *dystrophin* cDNA.[15] However, further improvements are needed to regulate the virus-associated host immune response before clinical trials can be performed.

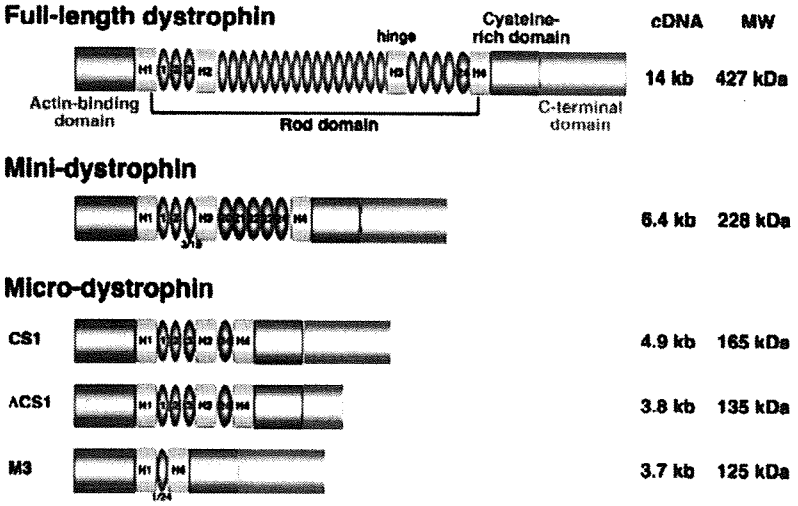


Fig. 2. Structures of full-length and truncated dystrophin. Helper-dependent adenovirus vector can package 14-kb of full-length dystrophin cDNA because of the large-sized deletion in its genome. A mini-dystrophin is cloned from a patient with Becker muscular dystrophy, which is caused by in-frame deletions resulting in the synthesis of partially functional protein. A series of truncated micro-dystrophin cDNAs harboring only four rod repeats with hinge 1, 2, and 4 (CS1); the same components, except that the C-terminal domain is deleted (delta CS1); or one rod repeat with hinge 1 and 4 (M3), are constructed to be packaged in the AAV vector.

A series of truncated *dystrophin* cDNAs containing rod repeats with hinge 1, 2, and 4 were constructed (Figure 2).[6] Although AAV vectors are too small to package the full-length *dystrophin* cDNA, AAV vector-mediated gene therapy using a rod-truncated *dystrophin* gene provides a promising approach.[16] The structure and, particularly, the length of the rod are crucial for the function of micro-dystrophin.[17] An AAV type 2 vector expressing micro-dystrophin (DeltaCS1) under the control of a muscle-specific MCK promoter was injected into the tibialis anterior (TA) muscles of dystrophin-deficient *mdx* mice,[18] and resulted in extensive and long-term expression of micro-dystrophin that exhibited improved force generation.

The impact of codon usage optimization on micro-dystrophin expression and function in the *mdx* mouse was assessed to compare the function of two different configurations of codon-optimized *micro-dystrophin* genes under the control of a muscle-restrictive promoter (Spc5-12).[19] Codon optimization of micro-dystrophin significantly increased micro-dystrophin mRNA and protein levels after intramuscular and systemic administration of plasmid DNA or rAAV8. By randomly assembling myogenic regulatory elements into synthetic promoter recombinant libraries, several artificial promoters were isolated whose transcriptional potencies greatly exceed those of natural myogenic and viral gene promoters.[20]

### 2.3. *Use of Surrogate Genes*

An approach using a surrogate gene would bypass the potential immune responses associated with the delivery of exogenous dystrophin. Methods to increase expression of utrophin, a dystrophin paralog, show promise as a treatment for DMD. rAAV6 harboring a murine codon-optimized micro-utrophin transgene was intravenously administered into adult *dko* mice to alleviate the pathophysiological abnormalities.[21] The paralogous gene efficiently acted as a surrogate for *dystrophin*. Myostatin is extensively documented as being a negative regulator of muscle growth. Systemic gene delivery of myostatin propeptide, a natural inhibitor of myostatin, enhanced body-wide skeletal muscle growth in both normal and *mdx* mice.[22] The delivery of various growth factors, such as insulin-like growth factor-I (IGF-I), has been successful in promoting skeletal muscle regeneration after injury.[23]

Matrix metalloproteinases (MMPs) are key regulatory molecules in the formation, remodeling and degradation of all extracellular matrix

(ECM) components in pathological processes. MMP-9 is involved predominantly in the inflammatory process during muscle degeneration.[24] In contrast, MMP-2 is associated with ECM remodeling during muscle regeneration and fiber growth.

## 3. AAV-mediated transduction of animal models

### 3.1. *Vector Production*

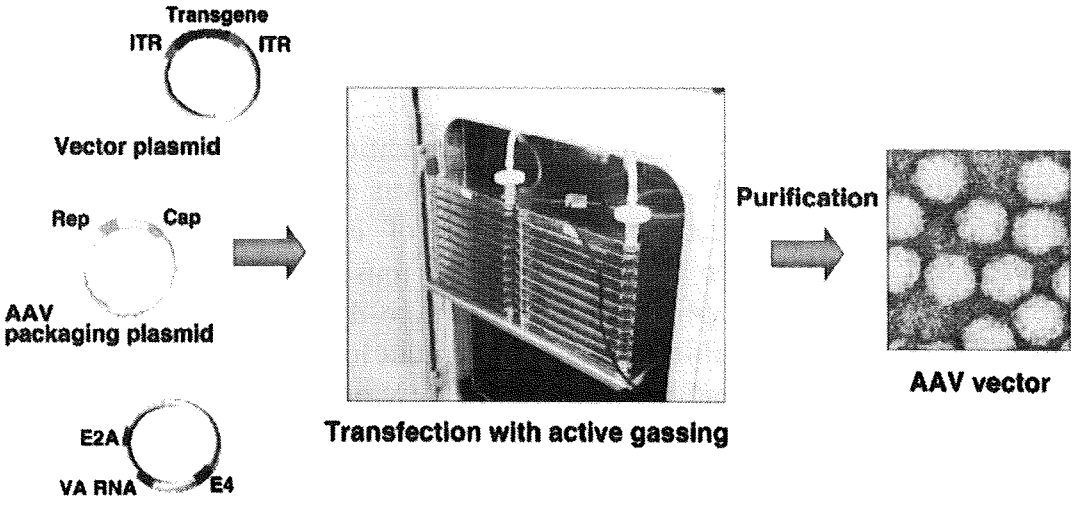


Fig. 3. A scalable triple plasmid transfection system using active gassing. When adenovirus helper plasmid is co-transfected into human embryonic kidney 293 cells along with a vector plasmid encoding the AAV vector and an AAV packaging plasmid harboring *rep-cap* genes, the AAV vector is produced as efficiently as when using adenovirus infection. A large-scale transduction method to produce AAV vectors with an active gassing system makes use of large culture vessels for labor- and cost-effective transfection in a closed system. Samples containing vector particles are further purified with a two-tier CsCl gradient or dual ion-exchange chromatography to obtain highly purified vector particles.

To gain acceptance as a medical treatment with a dose of over $1x10^{13}$ genome copies (g.c.)/kg body weight, AAV vectors require a scalable and economical production method. A production protocol of AAV vectors in the absence of a helper virus[25] is widely employed for triple plasmid transduction of human embryonic kidney 293 cells.[4] The adenovirus regions that mediate AAV vector replication (namely, the VA, E2A and E4 regions) were assembled into a helper plasmid. When this helper plasmid is co-transfected into human embryonic kidney 293 cells along with plasmids encoding the AAV vector genome and *rep-cap* genes, the AAV vector is produced as efficiently as when using

adenovirus infection (Figure 3). Importantly, contamination of most adenovirus proteins can be avoided in AAV vector stock made by this helper virus-free method. Samples containing vector particles are further purified with a two-tier CsCl gradient or dual ion-exchange chromatography to obtain highly purified vector particles.[4]

Despite improvements in vector production, including the development of packaging cell lines expressing Rep/Cap, and of methods that induce the expression and regulation of Rep/Cap,[26] maintaining such cell lines remains difficult, as the early expression of Rep proteins is toxic to cells. A scalable method, using active gassing and large culture vessels, was developed to transfect rAAV in a closed system, in a labor- and cost-effective manner.[27] This vector production system achieved a yield of more than $5x10^{13}$ g.c./flask by improving gas exchange to maintain the physiological pH in the culture medium. Recent developments also suggest that AAV vector production in insect cells would be compatible with current good manufacturing practice production on an industrial scale.[28]

### 3.2. *Animal Models for the Gene Transduction Study*

Dystrophin-deficient canine X-linked muscular dystrophy was found in a golden retriever with a 3' splice-site point mutation in intron 6.[29] The clinical and pathological characteristics of dystrophic dogs are more similar to those of DMD patients than are those of *mdx* mice. A beagle-based model of canine X-linked muscular dystrophy, which is smaller and easier to handle than the golden retriever-based muscular dystrophy dog (GRMD) model, has been established in Japan, and is referred to as $CXMD_J$.[30] The limb and temporal muscles of $CXMD_J$ are affected by two-months-old, which is the age corresponding to the second peak of serum creatine kinase.

Interestingly, we found extensive lymphocyte-mediated immune responses to rAAV2-*lacZ* after direct intramuscular injection into $CXMD_J$ dogs, despite successful delivery of the same viral construct into mouse skeletal muscle.[31] In contrast to rAAV2, rAAV8-mediated transduction of canine skeletal muscles produced significantly higher transgene expression with less lymphocyte proliferation than rAAV2.[32]

It is increasingly important to develop strategies to treat DMD that consider the effect on cardiac muscle. The pathology of the conduction system in $CXMD_J$ was analyzed to establish the therapeutic target for DMD.[33] Although dystrophic changes of the ventricular

myocardium were not evident at the age of 1 to 13 months, Purkinje fibers showed remarkable vacuolar degeneration when dogs were as young as four-months-old. Furthermore, degeneration of Purkinje fibers was coincident with overexpression of Dp71 at the sarcolemma. The degeneration of Purkinje fibers could be associated with the distinct deep Q waves present in ECGs and the fatal arrhythmias seen in cases of dystrophin deficiency.[33]

### 3.3. *Immunological Issues of rAAV*

Neo-antigens introduced by AAV vectors evoke significant immune reactions in DMD muscle, since increased permeability of sarcolemma allows leakage of the transgene products from the dystrophin-deficient muscle fibers.[34] rAAV2 transfer into skeletal muscles of normal dogs resulted in low and transient expression, together with intense cellular infiltration, and the marked activation of cellular and humoral immune responses.[31] Furthermore, an *in vitro* interferon-gamma release assay showed that canine splenocytes respond to immunogens or mitogens more strongly than do murine splenocytes. In fact, co-administration of immunosuppressants, cyclosporine (CSP) and mycophenolate mofetil (MMF) improved rAAV2 transduction. The AAV2 capsids can induce a cellular immune response via MHC class I antigen presentation with a cross-presentation pathway, and rAAV2 has also been proposed to have an effect on human dendritic cells (DCs). In contrast, other serotypes, such as rAAV8, induced T-cell activation to a lesser degree.[32] Immunohistochemical analysis revealed that the rAAV2-injected muscles showed higher rates of infiltration of $CD4^+$ and $CD8^+$ T lymphocytes in the endomysium than the rAAV8-injected muscles.[32]

Resident antigen-presenting cells, such as DCs, myoblasts, myotubes and regenerating immature myofibers, might play a role in the immune response. A recent study also showed that mRNA levels of MyD88 and co-stimulating factors, such as CD80, CD86 and type I interferon, are elevated in both rAAV2- and rAAV8-transduced dog DCs *in vitro*.[32] A brief course of immunosuppression with a combination of anti-thymocyte globulin (ATG), CSP and MMF was effective in permitting AAV6-mediated, long-term and robust expression of a canine micro-dystrophin in the skeletal muscle of a dog DMD model.[35]

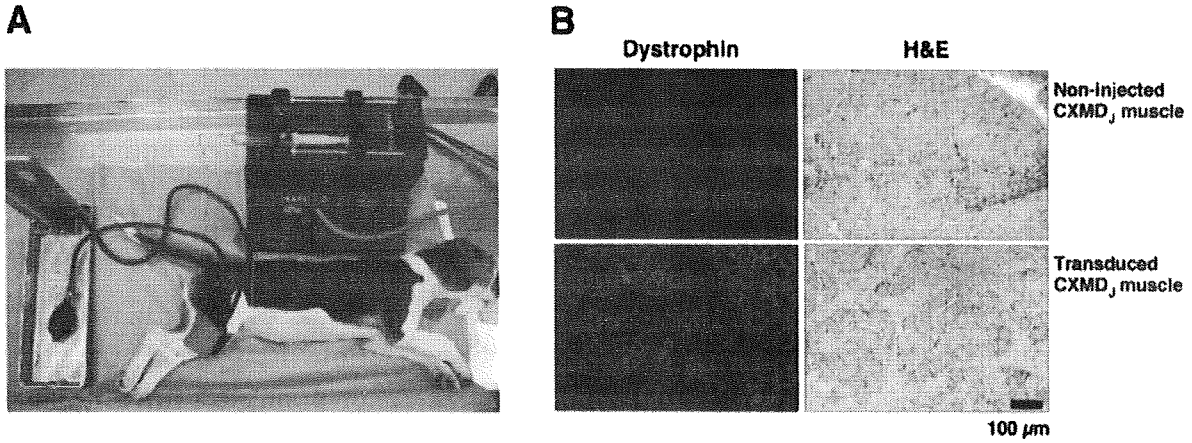


Fig. 4. Intravascular vector administration by limb perfusion. (A) A blood pressure cuff is applied just above the knee of an anesthetized CXMD$_J$ dog. A 24-gauge intravenous catheter is inserted into the lateral saphenous vein, connected to a three-way stopcock, and flushed with saline. With a blood pressure cuff inflated to over 300 mmHg, saline (2.6 ml/kg) containing papaverine (0.44 mg/kg, Sigma-Aldrich, St. Louis, MO) and heparin (16 U/kg) is injected by hand over a 10 second period. The three-way stopcock is connected to a syringe containing rAAV8 expressing micro-dystrophin (1 x $10^{14}$ vg/kg, 3.8 ml/kg). The syringe is placed in a PHD 2000 syringe pump (Harvard Apparatus, Edenbridge, UK). Five minutes after the papaverine/heparin injection, rAAV8 is injected at a rate of 0.6 ml/sec. (B) Administration of rAAV8-micro-dystrophin by limb perfusion produces extensive transgene expression in the distal limb muscles of CXMD$_J$ dogs without obvious immune responses at four weeks after injection.

### 3.4. *Intravascular Vector Administration by Limb Perfusion*

Although recent studies suggest that vectors based on AAV are capable of body-wide transduction in rodents, translating this finding into large animals remains a challenge. Intravascular delivery can be performed as a form of limb perfusion, which might bypass the immune activation of DCs in the injected muscle.[32] We performed limb perfusion-assisted intravenous administration of rAAV8-lacZ into the hind limb of normal dogs and rAAV8-micro-dystrophin into the hind limb of CXMD$_J$ dogs (Figure 4).[32] Administration of rAAV8-micro-dystrophin by limb perfusion produced extensive transgene expression in the distal limb muscles of CXMD$_J$ dogs without obvious immune responses for as long as eight weeks after injection.

### 3.5. *Global Muscle Therapies*

In comparison with fully dystrophin-deficient animals, targeted transgenic repair of skeletal muscle, but not cardiac muscle,

paradoxically elicits a five-fold increase in cardiac injury and dilated cardiomyopathy.[36] Because the dystrophin-deficient heart is highly sensitive to increased stress, increased activity by the repaired skeletal muscle provides the stimulus for heightened cardiac injury and heart remodeling. In contrast, a single intravenous injection of AAV9 vector expressing micro-dystrophin efficiently transduces the entire heart in neonatal *mdx* mice, thereby ameliorating cardiomyopathy.[37]

Since a number of muscular dystrophy patients can be identified through newborn screening, neonatal transduction may lead to an effective early intervention in DMD patients. After a single intravenous injection, robust skeletal muscle transduction with AAV9 vector throughout the body was observed in neonatal dogs.[38] Systemic transduction was achieved in the absence of pharmacological intervention or immune suppression and lasted for at least six months, whereas cardiac muscle was barely transduced in the dogs.

## 4. Safety and Potential Impact of Clinical Trials

The initial clinical studies lay the foundation for future studies, providing important information about vector dose, viral serotype selection, and immunogenicity in humans. The first virus-mediated gene transfer for muscle disease was carried out for limb-girdle muscular dystrophy type 2D using rAAV1. The study, consisting of intramuscular injection of virus into a single muscle, was discharged to establish the safety of this procedure in phase I clinical trials.[39] The first clinical gene therapy trial for DMD began in March 2006.[39] This was a Phase I/IIa study in which an AAV vector was used to deliver micro-dystrophin to the biceps of boys with DMD. The study was conducted on six boys with DMD, each of whom was transduced with mini-dystrophin genes in a muscle of one arm in the absence of serious adverse events.

While low immunogenicity was considered a major strength supporting the use of rAAV in clinical trials, a number of observations have recently provided a more balanced view of this procedure.[40] An obvious barrier to AAV transduction is the presence of circulating neutralizing antibodies that prevent the virion from binding to its cellular receptor.[41] This potential threat can be reduced by prescreening patients for AAV serotype-specific neutralizing antibodies or by performing procedures such as plasmapheresis before gene transfer. Another challenge recently revealed is the development of a cell-mediated cytotoxic T-cell (CTL) response to AAV capsid peptides. In the human

factor IX gene therapy trial in which rAAV was delivered to the liver, only short-term transgene expression was achieved and levels of therapeutic protein declined to baseline levels 10 weeks after vector infusion.[40] This was accompanied by elevation of serum transaminase levels and a CTL response toward specific AAV capsid peptides. To overcome this response, transient immunosuppression may be required until AAV capsids are completely cleared. Additional findings suggest that T-cell activation requires AAV2 capsid binding to the heparan sulfate proteoglycan (HSPG) receptor, which would permit virion shuttling into a DC pathway, as cross-presentation.[42] Exposure to vectors from other AAV clades, such as AAV8, did not activate capsid-specific T-cells.

## 5. Challenges and limitations of related strategies

### 5.1. *Design of Read-through Drugs*

To suppress premature stop codon mutations, treatments involving aminoglycosides and other agents have been attempted. PTC124, a novel drug capable of suppressing premature termination and selectively inducing ribosomal read-through of premature, but not normal, termination codons, was recently identified using nonsense-containing reporters.[43] The selectivity of PTC124 for premature termination codons, its oral bioavailability and its pharmacological properties indicate that this drug may have broad clinical potential for the treatment of a large group of genetic disorders with limited or no therapeutic options.

### 5.2. *Modification of mRNA Splicing*

By inducing the skipping of specific exons during mRNA splicing, antisense compounds against exonic and intronic splicing regulatory sequences were shown to correct the open reading frame of the DMD gene and thus to restore truncated yet functional dystrophin expression *in vitro*.[44] Intravenous infusion of an antisense phosphorothioate oligonucleotide created an in-frame *dystrophin* mRNA via exon skipping in a 10-year-old DMD patient possessing an out-of-frame exon 20 deletion of the *dystrophin* gene.[45] Moreover, the adverse-event profile and local dystrophin-restoring effect of a single intramuscular injection of an antisense 2'-O-methyl phosphorothioate oligonucleotide, PRO051, in patients with DMD were explored.[46] Four patients received a dose of 0.8 mg of PRO051 in the TA muscle. Each patient showed specific

skipping of exon 51 of dystrophin in 64 to 97% of myofibers, without clinically apparent adverse side effects.

The efficacy and toxicity of intravenous injection of stable morpholino phosphorodiamidate (morpholino)-induced exon skipping were tested using $CXMD_J$ dogs, and widespread rescue of dystrophin expression to therapeutic levels was observed.[47] Furthermore, a morpholino oligomer with a designed cell-penetrating peptide can efficiently target a mutated *dystrophin* exon in cardiac muscles.[48]

Long-term benefits can be obtained through the use of viral vectors expressing antisense sequences against regions within *dystrophin* gene. The sustained production of dystrophin at physiological levels in entire groups of muscles as well as the correction of muscular dystrophy were achieved by treatment with exon-skipping AAV1-U7.[49]

### 5.3. *Ex Vivo Gene Therapy*

Transplantation of genetically corrected autologous myogenic cells is a possible treatment for DMD. Freshly isolated satellite cells transduced with lentiviral vectors expressing micro-dystrophin were transplanted into the TA muscles of *mdx* mice, and these cells efficiently contributed to the regeneration of muscles with micro-dystrophin expression at the sarcolemma.[50] Mesoangioblasts are vessel-associated stem cells and might be candidates for future stem cell therapy for DMD.[51] Intra-arterial delivery of wild-type canine mesoangioblasts resulted in the extensive recovery of *dystrophin* expression, normal muscle morphology and function in the GRMD. Multipotent mesenchymal stromal cells (MSCs) are less immunogenic and have the potential to differentiate and display a myogenic phenotype.[52]

## 6. Future perspectives

### 6.1. *Pharmacological Intervention*

The use of a histone deacetylase (HDAC) inhibitor would likely enhance the utility of rAAV-mediated transduction strategies in the clinic.[53] In contrast to adenovirus-mediated transduction, the improved transduction with rAAV induced by the HDAC inhibitor is due to enhanced transgene expression rather than to increased viral entry. The enhanced transduction was proposed to be related to the histone-associated chromatin form of the rAAV concatemer in transduced cells. Since various HDAC inhibitors are currently being tested in clinical trials for

many diseases, the use of such agents in rAAV-mediated DMD gene therapy is theoretically and practically reasonable.

### 6.2. *Capsid modification*

A DNA shuffling-based approach for developing cell type-specific vectors is an intriguing possibility to achieve altered tropism. Capsid genomes of AAV serotypes 1-9 were randomly reassembled using PCR to generate a chimeric capsid library.[54] A single infectious clone (chimeric-1829) containing genome fragments from AAV1, 2, 8, and 9 was isolated from an integrin minus hamster melanoma cell line previously shown to have low permissiveness to AAV.

## 7. Conclusions and Outlook

DMD remains an untreatable genetic disease that severely limits motility and life expectancy in affected children. The systemic delivery of rAAV to transduce truncated dystrophin is predicted to ameliorate the symptoms of DMD patients in the future. To translate gene transduction technologies into clinical practice, development of an effective delivery system with improved vector constructs as well as efficient immunological modulation must be established. A novel protocol that considers all of these issues would help improve the therapeutic benefits of DMD gene therapy.

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疾　患　編

# 17. 筋ジストロフィー，多発筋炎

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**Key words**　Duchenne型筋ジストロフィー，多発筋炎，皮膚筋炎

**要　点**

①Duchenne型筋ジストロフィー（DMD）の確定診断は，遺伝学的検査あるいは筋生検により行う。
②DMDの骨格筋および呼吸機能の改善を目的にステロイド投与を行う。
③DMDでは，心不全と呼吸障害を中心とした全身管理が重要である。
④多発筋炎・皮膚筋炎は，ステロイド，免疫抑制剤，免疫グロブリンにより加療を行う。
⑤多発筋炎・皮膚筋炎は，悪性腫瘍や間質性肺炎の合併に注意する。

**重要ポイント**

①筋ジストロフィーは，骨格筋の壊死・変性を主病変とし，臨床的には進行性の筋力低下をみる遺伝性の疾患である。筋ジストロフィーのうち，もっとも頻度が高く重症の経過をとるDuchenne型筋ジストロフィーを中心に，呼吸および循環管理に加えて，ステロイド，アンジオテンシン変換酵素阻害薬，交感神経β受容体遮断薬などにより筋障害の改善が試みられている。
②後天性筋疾患の代表である特発性炎症性ミオパチーは，多発筋炎，皮膚筋炎，封入体筋炎に分類される。特に多発筋炎は横紋筋のびまん性炎症性筋疾患であり，特徴的な皮疹を呈するものは皮膚筋炎という。成人に比較的多くみられ，ステロイド，免疫抑制剤，免疫グロブリンが筋力低下に奏効する場合が多いが，悪性腫瘍や間質性肺炎の合併例は予後不良である。

## A Duchenne型筋ジストロフィー（Duchenne muscular dystrophy：DMD）

筋ジストロフィーは「骨格筋の壊死・変性を主病変とし，臨床的には進行性の筋力低下をみる遺伝性の疾患である」と定義される。筋ジストロフィーのうち，もっとも頻度が高く重症の経過をとるDuchenne型筋ジストロフィー（DMD）は，ジストロフィン遺伝子（Xp21.2）の変異により，骨格筋膜の安定性に重要なジストロフィンが欠損す

ることで発症する。ジストロフィンの欠損が不完全な場合はベッカー型筋ジストロフィー（Becker muscular dystrophy：BMD）の表現型をとる。DMDは，X染色体連鎖遺伝形式をとり，新生男児3,500人に1人の割合で発症する。

## 1．臨床像

2～5歳前後で転びやすい，歩行が遅いなどの症状で気づかれることが多いが，高CK血症を偶発的に指摘され診断に至ることもある。特徴的な登はん性起立（Gowers徴候）を呈する。病初期には明らかな筋萎縮は認めず，腓腹部や舌などの筋肥大を示す場合が多いが，徐々に近位筋優位の筋力低下が進行して歩行は動揺性となり，12歳までに歩行不能となり車椅子生活に移行する。前後して脊柱側彎や関節拘縮の出現をみる。13歳前後で座位の保持も困難となる。呼吸筋の筋力低下のため10歳後半に呼吸不全が生じ，次第に心機能の低下も出現する。主として呼吸管理の進歩により，平均死亡年齢は過去20年で10年程度延長し，30歳前後になった。現在の死因は主に心不全および呼吸不全である。

## 2．検査所見

### 1）血液生化学検査

乳児期より著明な高CK血症（20,000～40,000 U/L），アルドラーゼなどの筋原性酵素の上昇をみるが，筋萎縮の進行とともに低下する。AST，ALT，LDHも上昇し，肝機能障害と誤る場合がある。

### 2）筋電図

随意収縮時に，低振幅・短持続時間の運動単位，運動単位の早期動員（early recruitment）がみられる。

### 3）画像検査

骨格筋CT，MRIでは5歳頃から大殿筋の脂肪置換を認める。10歳以降では大腿四頭筋（特に大腿直筋），大内転筋と大腿二頭筋，傍脊柱筋を中心に近位筋優位に筋容積の減少や脂肪置換が顕著となるが，薄筋と縫工筋は比較的保たれる（selectivity pattern）。

### 4）遺伝学的検査

他の検査所見からDMD/BMDの可能性が疑われ，臨床的および遺伝医学的に有用と考えられる場合に実施を検討する。遺伝学的検査は，生涯変化しない個人の重要な遺伝学的情報を扱うため，担当医師から被験者（保護者）に対して，検査を行う意義，利点と限界，その結果が家族や親族に及ぼす影響について十分説明し，書面による同意を得た上で，遺伝子異常が診断されたときの支援まで準備して実施されるべきである。検査実施前後に遺伝カウンセラーが遺伝カウンセリングを実施することが望ましい。

最近，遺伝子変異が確立した男性のDMD/BMD患者を対象に，臨床試験/治験の実施を目的とした筋ジストロフィー患者登録サイトの運用が開始された（Remudy：registry of muscular dystrophy. http://www.remudy.jp/index.html）。登録に際しては，全例にmultiplex ligation-dependent probe amplification（MLPA）法によるスクリーニング検査を行い（保険診療），必要に応じてシークエンス解析，筋生検を実施する。

### 5）筋生検

筋病理では，筋の壊死・変性，再生線維の混在，筋線維の大小不同，結合織の増生

がみられる。免疫組織化学染色では，DMDの筋細胞膜はジストロフィンをほぼ完全に欠損するが，BMDの細胞膜はまだら状（faint and patchy）に染色される。

### 3．治療方針

DMDに対するステロイド投与の有効性に関して，筋力の増強あるいは維持と呼吸機能の改善がランダム化比較対照試験により証明されている。5～15歳の症例ではプレドニゾロン（プレドニン®）0.75 mg/kg/日の連続投与が治療の第一選択である。体重増加などの副作用の面から投与量の減量が望ましい場合には，0.5 mg/kg/日に減量し，3～4カ月でさらに0.3 mg/kg/日へと減量する。

【心不全】

定期的に脳性ナトリウム利尿ペプチド（brain natriuretic peptide：BNP）の測定や心エコーを施行し，左室駆出率40％以下（BNP 20～60 pg/mL）で，アンジオテンシン変換酵素阻害薬（レニベース®，2.5 mg/日から開始し漸増），交感神経β受容体遮断薬（アーチスト®，1.25 mg/日から開始し漸増。上限は10 mg/日）を開始する。心筋障害が進行した際は拡張型心筋症の心不全に準じ，強心薬，利尿薬も加える。

【呼吸障害】

定期的な$SpO_2$，％VC，ピークカフフロー，終末呼気炭酸ガス濃度の測定が重要である。開始時期は，低酸素に基づく症状がある場合，睡眠時に$SpO_2$低下がある場合，VCが1L（あるいは％VCが20％）を下回る時期の前後，$PaCO_2$が55 Torr以上であれば夜間に非侵襲的陽圧換気療法（Noninvasive Positive Pressure Ventilation：NPPV）を開始する。病状，病態に応じて昼間にもNPPVを追加する。排痰障害にはカフレーター（Mechanical In-Exsufflator：MI-E）や肺内パーカッション換気療法（IPV）も有効である。

### 4．患者指導とリハビリテーション

早期より側彎と関節拘縮の予防に努め，必要に応じて装具，コルセットを作製する。最大強制吸気量維持のため呼吸訓練を行い，舌咽頭呼吸の習得を試みる。側彎は外科的治療も含め積極的に治療する。過度の痩せは消化管機能を低下させるため栄養指導が大切である。

### 5．根本的治療開発の動向

現在DMDに対して，PTC124によるリード・スルー療法，ES/iPS細胞や筋芽細胞の移植治療，ウイルスベクターによる遺伝子治療などの開発が進められている。当研究部では，これまでアンチセンス・モルフォリノを用いたエクソン・スキッピング療法の前臨床試験を行ってきた。この成果を踏まえて，DMDを対象にしたエクソン51スキッピングの臨床治験を実施する準備を進めている。

## B 多発筋炎（polymyositis：PM），皮膚筋炎（dermatomyositis：DM）

後天性筋疾患の代表である特発性炎症性ミオパチーは，多発筋炎，皮膚筋炎，封入体筋炎に分類される。特に多発筋炎は横紋筋のびまん性炎症性筋疾患であり，特徴的な皮疹を呈するものは皮膚筋炎という。多発筋炎・皮膚筋炎の有病率は人口10万あた

り約6人と推定される。男女比は女性が約2倍と多い。発症年齢の約半数は40～60歳である。

## 1. 病因

発症には自己免疫機序が関与する。多発筋炎では，筋線維・間質・血管周囲にマクロファージ，$CD8^+$T細胞が浸潤し，筋線維内のカルパインなどのタンパク分解酵素を活性化する結果，筋線維は壊死する（細胞性免疫）。皮膚筋炎では，主に筋周膜の血管周囲や間質にB細胞，$CD4^+$T細胞（ヘルパーT細胞）が浸潤する（液性免疫）。筋内微小血管の内皮細胞が傷害される結果，循環障害による筋束周囲萎縮が生じる。

## 2. 臨床像

急性ないし亜急性（数週～数ヵ月）に進行する。初発症状は，四肢近位筋・頸筋・体幹の筋力低下，筋痛，関節痛がみられることが多い。遠位筋力の低下は遅れて生ずる。進行例では筋萎縮を認めることがある。嚥下障害が生じることがあるが，構音障害を伴うことは少ない。びまん性間質性肺炎，肺線維症，心筋炎をしばしば合併する。自然寛解や増悪を繰り返しつつ徐々に進行し，5年生存率は約75％である。皮膚筋炎は多発筋炎と類似した臨床像を呈するが，ゴットロン徴候（指関節伸側で肥厚した紅斑），ヘリオトロープ疹（上眼瞼の紫紅色の浮腫性紅斑）を伴うことを特徴とする。皮膚筋炎の悪性腫瘍合併頻度は約20％であり，多発筋炎と比べて2.1～6.5倍高い。女性では，乳癌・卵巣腫瘍，男性では肺癌・消化器癌・前立腺癌が多い。女性の悪性腫瘍合併率は男性の約2倍で，50歳以上は高い。

## 3. 検査所見

### 1) 血液生化学検査

活動期には血清CK値は正常値の約10倍に上昇し，ミオグロビン値も上昇する。アルドラーゼ，AST，ALT，LDH，％クレアチン尿（尿中クレアチン/尿中クレアチン＋尿中クレアチニン）が上昇し，活動性の指標判定に有用である。

### 2) 筋電図

随意収縮時には，低振幅・短持続時間の運動単位，運動単位の早期動員（early recruitment）がみられる。刺入電位は亢進していることが多い。安静時には線維性収縮電位，陽性鋭波を認める。

### 3) 画像検査

急性期の骨格筋MRIは，STIR（Short TI Inversion Recovery）法および脂肪抑制T2強調画像では，病変は多巣性あるいはびまん性の高信号を示す。進行例は，筋萎縮およびT1強調画像で高信号を示す。

### 4) 筋生検

筋束内の周辺・筋線維の内部・血管周囲に$CD8^+$T細胞やマクロファージの浸潤像，筋線維の変性と再生，結合織の増生を認める。特に皮膚筋炎では血管周囲の細胞浸潤が主体であり，筋束周囲萎縮が認められることが多い。

### 5) 自己抗体

抗Jo-1抗体は肺線維症の合併のある多発筋炎の50％，皮膚筋炎の20％に認められる。抗シグナル認識粒子（SRP）抗体は筋炎と心障害を伴う急性発症の重症皮膚筋炎および多発筋炎の5％に検出される。皮膚筋炎に特異的な抗Mi-2抗体は35％で検出され，抗

p155抗体は悪性腫瘍合併例で高率とされる。その他の膠原病を合併するオーバーラップ（重複）症候群では，抗PM-1抗体（強皮症），抗Ku抗体（強皮症・全身性エリテマトーデス），抗nRNP抗体（混合性結合組織病）が陽性となることがある。

## 4. 診断

BohanとPeterの診断基準や皮膚筋炎・多発筋炎の改訂診断基準（厚生省特定疾患自己免疫疾患調査研究班平成4年度研究報告，pp25-28，1993）が汎用される。

## 5. 治療方針

多発筋炎あるいは皮膚筋炎の確定診断後は，プレドニン®1～1.5 mg/kg/日を1～2ヵ月間連日投与する。筋力の改善，血清CK値の減少がみられれば2週間に10％の割合でプレドニン®を減量し，2～3年程度は維持療法を行う。筋症状は早期治療例ほど回復がよい。改善がないときは同量を1～2ヵ月間投与するか，ステロイドパルス療法を2～3クール行う（メチルプレドニゾロン1 g/回の3日間連続投与）。効果がない場合はメトトレキサート（メソトレキセート®，5～25 mg/週，経口あるいは筋肉内投与），アザチオプリン（イムラン®，50～100 mg/日，経口投与）などを併用する。ステロイド，免疫抑制薬の無効例では，追加療法として免疫グロブリン療法を併用する。

## 6. 治療のポイント

筋症状増悪時には筋炎の再燃か，ステロイドミオパチーの合併かの鑑別が重要である。血清CK値上昇，筋電図で線維性収縮電位や陽性鋭波の出現頻度が上昇した場合には再燃を疑う。ステロイドミオパチーは，プレドニン内服を4週間以上続けた場合に発症し，下肢近位筋優位の筋力低下・筋萎縮を呈するが，顔面筋および頸部伸展筋は保たれ，血清CK値は低下する。

## 7. 患者指導とリハビリテーション

急性期は等尺性収縮以外の運動は避ける。安定後は誤嚥性肺炎，廃用性筋萎縮，関節拘縮予防のための理学療法を早期に開始する。

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# 5. 骨格筋

骨格筋は傷害を受けると再生する（図1）．筋傷害シグナルにより骨格筋特異的幹細胞である筋衛星細胞（muscle satellite cells）が活性化され，分裂・増殖し，やがてお互いに融合，あるいは既存の筋線維と融合して筋線維を再生する．デュシェンヌ型筋ジストロフィー（Duchenne muscular dystrophy；DMD）等の重篤な遺伝性筋疾患に対して筋・幹細胞を移植する再生医療が期待されているが，その確立のためには，筋組織の再生がどのように制御されているかを理解することが重要である．

## a. 骨格筋衛星細胞

筋衛星細胞は筋基底膜と筋線維の間にある単核の細胞で1961年にAlexander Mauroによって初めてその存在を記載された．通常，細胞周期のG0の状態にあるが，筋傷害時に活性化され，増殖して筋線維を再生する．生直後は骨格筋組織の中の核の30％程度が筋衛星細胞の核であるが，成体になると5％程度とほぼ一定になる．体幹と四肢の骨格筋の発生学的な起源は沿軸中胚葉由来の体節であり，その中に形成されるdermomyotomeに出現するPax3，Pax7陽性の筋前駆細胞（muscle progenitor cells）が増殖し，やがてMyf5，MyoD等の筋分化制御遺伝子を発現して筋芽細胞（myoblast）となり，次に増殖を止め，融合して，筋線維を形成する．筋衛星細胞はその過程で派生してく

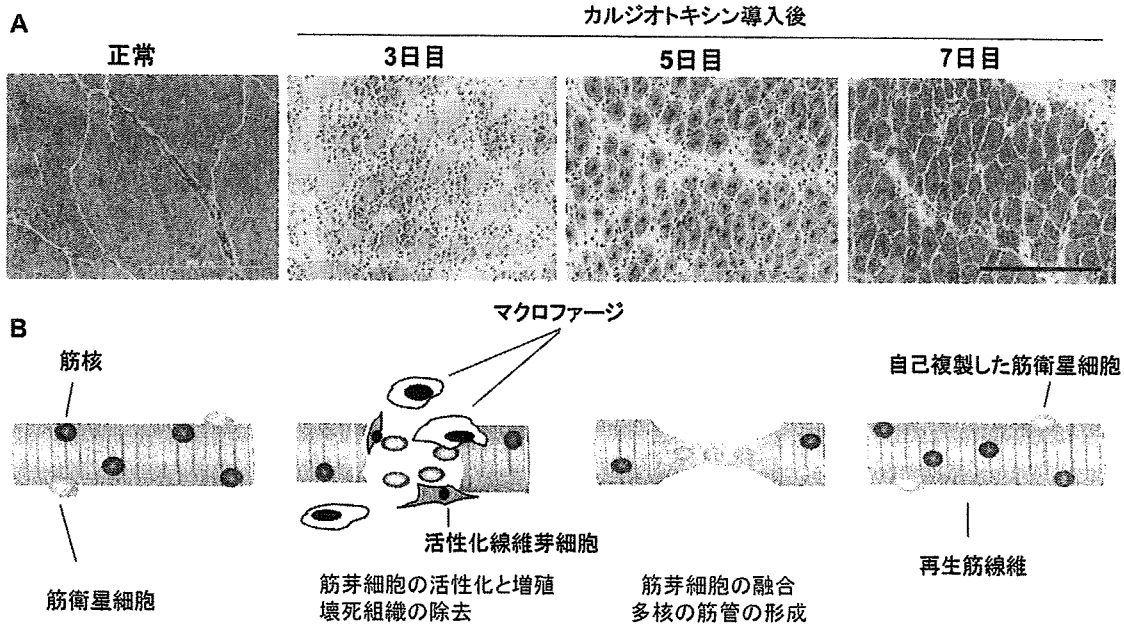


図1 骨格筋の再生

A） C57Bl/6マウス骨格筋に蛇毒であるカルジオトキシンを導入して筋傷害を引き起こした後の組織修復過程を示す．ヘマトキシリン・エオジン染色．スケールバー：200ミクロン．

B） 骨格筋特異的幹細胞である筋衛星細胞は，静止期の状態では筋基底膜と筋線維の間に存在するが，筋傷害時には活性化し，増殖する（筋芽細胞）．やがてお互いに融合し，あるいは既存の筋線維と融合して筋再生が完了する．この過程には好中球やマクロファージ等による壊死組織の貪食機能が重要である．活性化した筋衛星細胞の一部は，元の筋衛星細胞の状態に戻る（自己複製）．