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RNAi-based Gene Therapy for Dominant Limb Girdle Muscular Dystrophies

Jian Liu and Scott Q. Harper*

Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital and
Department of Pediatrics, The Ohio State University College of Medicine, Columbus, OH 43205,
USA

Abstract

Limb Girdle Muscular Dystrophy (LGMD) refers to a group of 25 genetic diseases linked by common clinical features, including wasting of muscles supporting the pelvic and shoulder girdles. Cardiac involvement may also occur. Like other muscular dystrophies, LGMDs are currently incurable, but prospective gene replacement therapies targeting recessive forms have shown promise in pre-clinical and clinical studies. In contrast, little attention has been paid to developing gene therapy approaches for dominant forms of LGMD, which would likely benefit from disease gene silencing. Despite the lack of focus to date on developing gene therapies for dominant LGMDs, the field is not starting at square one, since translational studies on recessive LGMDs provided a framework that can be applied to treating dominant forms of the disease. In this manuscript, we discuss the prospects of treating dominantly inherited forms of LGMD with gene silencing approaches.

Keywords

AAV; dominant myopathy; gene therapy; LGMD; limb-girdle muscular dystrophy; miRNA; RNAi; RNA interference

1. INTRODUCTION

The Limb Girdle Muscular Dystrophies (LGMD) are a group of genetic disorders characterized predominantly by progressive wasting and weakness of proximal limb girdle muscles, including pelvic, shoulder, upper arm and thigh muscles [1–3]. Despite these common features, LGMD pathologies are highly heterogeneous [2]. The onset of LGMD symptoms usually varies from early childhood to late adulthood, and the progression rate and distribution of weakness and wasting also varies considerably among individuals and genetic subtypes. As a result, some LGMD patients may be only mildly affected, while

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*Address correspondence to this author at the Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital and Department of Pediatrics, The Ohio State University College of Medicine, Columbus, OH 43205, USA; Tel: (614) 355-2893; scott.harper@nationwidechildrens.org.

CONFLICT OF INTEREST

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others become wheelchair dependent, develop cardiomyopathy, or suffer potentially life-threatening respiratory muscle weakness. Like other muscular dystrophies, there are currently no interventions that slow or reverse the course of any form of LGMD. Nevertheless, important molecular genetics studies have led to the identification of numerous disease genes underlying various forms of LGMD, and this fundamental knowledge has opened the door to animal model creation and a greater understanding of the molecular mechanisms of LGMD and muscular dystrophy in general. In parallel, significant progress has been made toward making muscle gene therapy and RNAi therapeutics feasible. This combined accumulation of knowledge and a scientific toolbox provide the necessary framework to begin developing RNAi-based gene therapies for dominant LGMDs.

In this review, we specifically focus on this class of disorders, and discuss the prospects for therapeutic gene silencing using the current state-of-the-art methods for RNAi and muscle-directed gene therapy.

2. CLASSIFICATION

Currently, the Online Mendelian Inheritance in Man (OMIM) database lists 24 different subtypes of LGMD based on clinical features and linkage to specific genetic loci (www.ncbi.nlm.nih.gov/omim). In addition, a new form of LGMD (LGMD2P) was recently described and is not yet listed in the OMIM database ([3], [4]). The disorders are first classified by inheritance pattern (autosomal dominant [LGMD1] and autosomal recessive [LGMD2] forms), followed by an alphabetical annotation specifying an underlying gene mutation or linkage to a chromosomal locus. Thus, the 8 dominant forms of LGMD are annotated LGMD1A-H and the 17 autosomal recessive ones denoted LGMD2A-Q. In this review, we will specifically focus on dominantly inherited LGMDs and discuss the prospects of RNAi-based gene silencing as a common therapeutic approach for these disorders.

2.1. Dominant LGMDs

There are 8 autosomal dominant LGMDs (LGMD1), but to date only 5 have been linked to specific gene mutations (LGMD1A-E). The phenotypic heterogeneity often seen in patients contributes to variable clinical diagnoses, and dominant LGMDs are therefore often allelic with other clinical disorders, including the myofibrillar myopathies (MFMs) or dilated cardiomyopathy (DCM).

LGMD1A—LGMD1A (OMIM #159000) is caused by missense mutations in MYOT (myotilin) [5–7], a gene encoding a structural Z-disc protein in striated muscle [8]. LGMD1A patients develop proximal limb weakness that may later progress to distal muscles, and patients may also show intramuscular myofibrillar aggregates and Z-disc abnormalities [5, 6]. The latter features are typical of the MFMs, and indeed myotilin mutations are sometimes associated with a form of MFM called spheroid body myopathy (OMIM #182920) [9]. Thus, myopathies related to MYOT mutations are often more generally referred to as myotilinopathies (OMIM #609200). To date, 11 different MYOT mutations have been identified in various familial pedigrees [5–7, 10–12]. One mutant

(T57I) was expressed in skeletal muscles of transgenic mice using the human skeletal actin (HSA) promoter, and animals developed progressive functional and histological features of LGMD1A, including intramuscular protein aggregation [13].

LGMD1B—This most common form of dominant LGMD (OMIM #159001) is caused by mutations in LMNA [14–18], which encodes four proteins (Lamin A, A 10, C and C2) by alternative splicing [19]. The predominant isoforms, lamin A and lamin C, are expressed in all post-mitotic cells and assemble into dimers and polymers to form the nuclear lamina [20]. As such, the lamins play important roles in nuclear stability, chromatin organization, gene expression and signal transduction. Reflective of these wide-ranging functions, LMNA mutations may have broad clinical effects that manifest as autosomal dominant (AD) or recessive (AR) disorders. Indeed, in addition to dominant LGMD1B, OMIM lists 10 other disease phenotypes associated with LMNA mutations, including AD and AR forms of Emery-Dreifuss muscular dystrophy (EDMD; OMIM #181350) [21, 22], AD congenital muscular dystrophy (OMIM #613205) [23, 24], AD dilated cardiomyopathy (CMD1A; OMIM #115200) [25, 26], AD familial partial lipodystrophy (FPLD2; OMIM #151660) [27–29], AR axonal Charcot-Marie-Tooth disease type 2B1 (CMT2B1; OMIM #605588) [30, 31], and premature aging syndromes such as AD Hutchinson-Gilford progeria syndrome (HGPS; OMIM #176670) [32] or AR mandibuloacral dysplasia (MAD; #248370) [33, 34]. Although several of these LMNA mutants have been modeled in mice, the resultant animals are considered models of EDMD, dilated cardiomyopathy, FPLD2, or progeria (Table 1). Thus, a faithful phenocopy of LGMD1B is still lacking.

LGMD1C—LGMD1C (OMIM #607801) is caused by a variety of dominant caveolin-3 (CAV3) mutations that often result in CAV3 protein deficiency in muscle biopsies, thereby suggesting that dominant negative pathogenic mechanisms underlie some severe, early onset forms of CAV3-related LGMD1 [35, 36]. Indeed, transgenic mice expressing a clinically derived CAV3 mutant (P104L) recapitulate CAV3 deficiency and severe limb girdle myopathy described in patients bearing the same mutation (Table 2) [37]. Nevertheless, heterogeneity exists within the caveolinopathies: some CAV3 mutations are associated with the typically milder allelic autosomal dominant disorder, Rippling Muscle Disease (RMD; OMIM #606072) [38, 39], or autosomal dominant hypertrophic cardiomyopathy [40, 41] and long QT syndrome [42, 43] (OMIM #192600 and #611818). Still other CAV3-related mutations produce relatively milder clinical manifestations (elevated serum creatine phosphokinase syndrome or hyperCKemia [44, 45]; OMIM #123320) or require homozygous inheritance (autosomal recessive forms of rippling muscle disease [46]).

LGMD1D—LGMD1D was first identified in 25 individuals of a 4-generation family suffering from adult-onset LGMD phenotypes as well as dilated cardiomyopathy [47]. The causative mutation was recently identified as a splice site mutation in the desmin gene on 2q35, and the disease is now officially classified as a desmin-related myofibrillar myopathy (OMIM #601419) [48].

LGMD1E—LGMD1E (OMIM #603511) is a progressive, late onset LGMD described now in at least 12 multi-generational families, 8 of which are Finnish. The disorder was formerly

classified as LGMD1D and is still listed as such by the HUGO Gene Nomenclature Committee (HGNC). This disorder was first linked to chromosome 7q in 1999 [49] and the underlying gene mutation was recently identified as a missense change in the DNAJB6 gene, which encodes a ubiquitously expressed chaperone protein [50]. There are no mouse models expressing mutant DNAJB6 proteins, but zebrafish injected with patient-derived cDNAs develop myopathic phenotypes [50].

LGMD1F—Currently, LGMD1F has only been described in a 5 generation Spanish family in which 32 members presented with characteristic proximal weakness in pelvic and shoulder girdle muscles [51]. As is typical of other LGMDs, phenotypic heterogeneity was evident in this cohort, including variability in age-at-onset (in some cases by several decades), and involvement of distal limb or respiratory muscles. Like LGMD1E, this disorder was linked to a distal region on chromosome 7q, but mapped to a more centromeric chromosomal marker, supporting that the two disorders arise from different genetic abnormalities.

LGMD1G—Like LGMD1F, this disorder has so far been described only in one family (from Brazil) [52]. Patients develop a mild, adult-onset limb girdle myopathy linked to a 7 Mb region on chromosome 4q21.

LGMD1H—LGMD1H is another late-onset, mild LGMD known to occur in only one multi-generational family, hailing from southern Italy [53]. The disease locus was mapped to the short arm of chromosome 3, which includes the CAV3 gene, although no CAV3 mutations have yet been identified as an underlying cause of LGMD1H.

3. GENE THERAPY

Molecular genetics emerged during the last century, and with it came the tools needed to decipher the molecular basis of disease. From this knowledge the notion arose, as early as 1972, that genetic disorders could be treated with gene therapy [54]. Conceptually the strategy was simple: replace bad genes with good ones in affected cells or tissues. Within a few years, the nascent gene therapy field began acquiring the tools required to put gene replacement strategies for recessive disease into practice. In contrast, feasible gene silencing tools for treating dominant diseases emerged only within the last decade (discussed below). Although gene therapy seemed straightforward in theory, in practice it proved much more challenging for a myriad of reasons [55], and the field underwent several decades' worth of growing pains to reach a point today where clinical gene therapy is becoming a reality. Indeed several successful strategies for a variety of diseases have been recently described (e.g. SCID [56], hemophilia [57], Leber's congenital amaurosis [LCA] [58–62]).

3.1. Muscle Gene Therapy

The gene therapy field is on a promising upward trajectory, and despite the successes mentioned above, it is perhaps obvious that different diseases require different strategies and no single “magic bullet” gene therapy approach can be applied to every disorder. Thus, the clinical successes seen in the retinal disorder LCA, for example, may not necessarily be informative for strategies targeting the muscular dystrophies. Indeed, LCA may be among

the most amenable diseases for gene therapy, while the muscular dystrophies are arguably among the most challenging.

In the LCA trials, remarkable improvements in the vision of clinically blind patients were seen following AAV-mediated delivery of a functional RPE65 gene via subretinal injection [59–62]. This groundbreaking work benefitted from several important factors, including that the gene product, RPE65, could be easily packaged and delivered to the eye using an AAV vector system with natural tropism to the affected retinal cells, and because the retina itself is arguably among the best tissues for gene delivery, as it is small, relatively superficial, and immune privileged. Thus, because of these advantages, a singly-injected dose of a small volume vector product ($1.5 - 4.8 \times 10^{10}$ total vector genomes in 150 – 300 microliter volume) was used to express RPE65 at therapeutic levels [61, 62].

In contrast, muscle is an exponentially larger target than the retina. Human beings have about 640 different skeletal muscles, as well as cardiac tissue, that may comprise up to ~40% of a person's total mass. Thus in an average 70 kg person, as much as 28 kg may be composed of muscle tissue, while the average eyeball weighs ~7.5 g, of which retinal cells only constitute a portion [63]. Simplistically these numbers suggest that humans have roughly 3,700 times more muscle than retinal mass. Thus, transducing this exponentially larger tissue target requires significantly more vector administered in larger volumes. Although the vector doses and administration volumes required to achieve complete transduction of human skeletal muscle are not experimentally known, we speculate that roughly 10^{15} AAV particles, administered in a significantly larger volume than used for retinal delivery (perhaps liters), may be required for global muscle delivery. Accompanying this theoretical 100,000 fold increase in dose is an additional cost component required to produce large amounts of virus. Another challenge involves delivering therapeutic vectors to a dystrophic muscle environment which may contain abundant immune cell infiltrates and could therefore be more prone to anti-treatment immune responses compared to vector administration in the retina.

Despite these challenges inherent in muscle-directed gene transfer, significant progress has been made in the field over the last two decades, including: identification of optimal vectors for muscle gene transfer (numerous myotropic AAV serotypes), improved large scale AAV vector production protocols, characterization of muscle specific promoters, and optimization of local and global vascular delivery paradigms. These advancements helped contribute to the recent demonstration of sustained therapeutic gene expression following intramuscular delivery of AAV- α -sarcoglycan to muscles of human LGMD2D patients in a pilot Phase I/II clinical trial [64–66]. Considering the considerable challenges standing in the way of muscle gene transfer just two decades ago, it is apparent that the field has achieved remarkable progress [67, 68]. Although hurdles still remain, the stage is now set for future successes [68]. Importantly, these muscle-directed gene therapy strategies now include delivery of RNAi triggers for treating dominant myopathies, like the Type 1 LGMDs.

3.2. RNAi Therapy for Dominant LGMD1

3.2.1 RNAi Therapy—Until recently, the muscle gene therapy field was primarily focused on treating recessive diseases using gene replacement, while therapies for dystrophies

caused by dominant mutations were largely unexplored by comparison. This disparity at least partly arose because the technologies existed to replace genes underlying recessive disease, but feasible mechanisms to silence dominant disease genes lagged behind. This has changed now with the emergence of RNA interference (RNAi) as a powerful tool to inhibit gene expression in a sequence-specific manner [69]. As such, RNAi has provided opportunities to develop new therapeutic strategies to silence disease genes underlying dominant LGMDs and other dominantly-inherited myopathies. RNAi technology possesses several therapeutic advantages, such as high potency, sequence specificity, and potentially less immunogenicity, because the “gene products” used to trigger RNAi are non-protein-coding, making RNAi-mediated gene therapy less likely to be hampered by potential host immune responses to encoded products.

Several classes of small RNAs are known to trigger RNAi processes in mammalian cells, including short (or small) interfering RNA (siRNA), and short (or small) hairpin RNA (shRNA) and microRNA (miRNA), which constitute a similar class of vector-expressed triggers [69, 70]. From a therapeutic standpoint, siRNA refers to chemically synthesized ~22 nt double-stranded RNA molecules that are delivered into cells using typical non-viral delivery mechanisms (e.g. liposomes). siRNAs are currently not the optimal system for triggering RNAi as a therapy for dominant LGMDs or other muscular dystrophies because these disorders likely require chronic suppression of their respective dominant alleles, and siRNAs are relatively unstable *in vivo* and single administration only achieves transient silencing effects [71]. Thus, achieving therapeutic long term silencing of a dominant disease gene would require repeated administration of the therapeutic siRNA to muscle. As non-viral nucleic acid delivery systems improve, this approach may someday be more feasible. Indeed, a transient system may hold advantages, since RNAi triggers have the potential to induce off-target effects [72, 73], and an siRNA-based system would allow dose alterations or halting of treatment if such unintended side effects should arise. In contrast, shRNA and miRNA are expressed *in vivo* from plasmid- or virus-based vectors and may thus achieve long term gene silencing with a single administration, for as long as the vector is present within target cell nuclei and the driving promoter is active [74]. Importantly, this vector-expressed approach leverages the decades-long advancements already made in the muscle gene therapy field, but instead of expressing protein coding genes, the vector cargo in RNAi therapy strategies are artificial shRNA or miRNA cassettes targeting disease genes-of-interest. From this standpoint, RNAi therapy for muscular dystrophy has been able to hit the ground running by coopting previously established vector systems and gene delivery procedures optimized initially for coding gene expression in muscle. As a result, in the past two years, well-established muscle gene therapy strategies were used to demonstrate pre-clinical proof-of-principle for RNAi-based gene therapy in animal models expressing candidate genes for dominant Facioscapulohumeral muscular dystrophy (FSHD) [75–77]. Importantly, these FSHD-directed methods can be modified to target genes underlying other dominant myopathies, including the LGMD1s [78].

3.1.2. RNAi Therapy for LGMD1—In general, the natural history of the LGMDs makes them good candidates for gene therapy. Specifically, the progressive nature of the disease may provide a window for therapeutic intervention when muscle tissue is still abundant and

amenable to correction. In addition, because the LGMDs primarily affect specific limbs, relatively limited interventions in a few muscles may profoundly benefit patients. This latter point has implications for the scale of vector production required per treatment, as well as the associated cost. Finally, there is recent precedence for safety and efficacy of AAV-mediated gene transfer to patients with a recessive form of LGMD [64–66], which therefore sets the stage for future clinical trials using similar gene delivery strategies, including those involving RNAi. Nevertheless, we are still at the emerging stages of RNAi-based gene therapy for dominant LGMDs. There are currently no published reports yet in this field of study, but the necessary tools and base of knowledge base have accumulated to the point where therapy development is now possible. Important assets already discussed in this review include disease gene identification, development of animal models, and proof-of-principle for RNAi-based gene therapy using well-established muscle gene transfer strategies originally developed for coding gene expression. In this section, we briefly discuss these current assets as they pertain to each LGMD1 disorder, and where additional work is needed.

LGMD1A: This disorder is ideal for demonstrating proof-of-principle for RNAi therapy of dominant LGMD. The underlying gene mutations (MYOT) are known; an excellent mouse model is available that recapitulates human LGMD1A pathologies [13]; and MYOT null mice develop normally and do not show any overt pathologies [79]. This last point is important because it has implications for ease of RNAi trigger design. Specifically, patients with autosomal dominant disorders possess one normal and one mutant copy of their specific myopathy-related gene. Simply reducing the mutant allele may be therapeutic. However, the normal alleles of many dominant disease genes encode essential proteins, and sufficient levels of the wild-type allele may be required to maintain some level of normal muscle function. Thus a gene silencing strategy that reduces expression of both mutant and normal alleles may be undesirable if reducing the wild-type copy counteracts any beneficial effects of disease allele suppression.

Because mammalian muscles seem to tolerate MYOT absence, and the dominant MYOT mutations have a gain-of-function effect in muscle, disease allele-specific silencing of MYOT is not required. Thus, RNAi triggers do not need to be designed to discriminate between mutant and normal MYOT alleles, which typically vary by only a single missense mutation. Although this is possible, it is much more challenging to accomplish [80]. LGMD1A is thus an ideal test case for RNAi therapy development for dominant muscular dystrophy.

LGMD1B: As discussed above, LMNA mutations cause a wide array of clinical pathologies, including LGMD1B, EDMD, and progeria. Mice containing a heterozygous null LMNA mutation do not develop obvious skeletal myopathy, but do show progressive cardiomyopathy that begins around 10 weeks [81–83]. Thus, skeletal muscles appear to require only 50% or less of wild-type LMNA to function normally, which suggests that disease allele-specific silencing would be beneficial for LGMD1B therapy. Nevertheless, although there are currently 11 different published animal models of laminopathies, none are true phenocopies of LGMD1B (Table 1). The model that most closely recapitulates

LGMD1B skeletal muscle pathology contains an H222P change knocked-in to the endogenous LMNA allele, and these animals only manifest disease phenotypes with H222P homozygosity [84]. This mouse therefore does not possess a normal LMNA gene copy, and cannot be used to test allele-specific silencing strategies in skeletal muscle. Further mouse model development is therefore required for targeting mutation involved in skeletal myopathy. However, one of the currently available models (M371K mice) [85] may be useful for testing the therapeutic effects of silencing a dominant LMNA mutation associated with EDMD-related cardiomyopathy since the mice possess both mutant and normal LMNA alleles, and cardiac muscle can be readily transduced by the same AAV systems used for skeletal muscle transduction. Alternatively, allele-specific silencing strategies for LMNA could be tested *in vitro*, or a vector-based model of skeletal muscle laminopathy could be developed for therapeutic testing, using a strategy similar to that published by Wallace, *et al.* [76].

LGMD1C: Like the laminopathies, LGMD1C may be amenable to disease allele-specific silencing, since heterozygous CAV3 null mice are normal, while homozygous animals develop progressive skeletal and cardiac myopathies [86, 87]. These knockout mouse data support that striated muscles can tolerate reduced wild-type CAV3 expression somewhere between 0 and 50% of normal levels. Importantly, there is a published mouse model expressing a CAV3 mutation under control of the MCK promoter that recapitulates phenotypes associated with LGMD1C, which is useful for testing RNAi-based strategies of CAV3 suppression [40, 88].

LGMD1D and E: The underlying genetic defects leading to these disorders were only recently described and no mouse models containing these mutations have yet been generated. Homozygous DNAJB6 null mice die at mid-gestation while heterozygous null animals are normal [89]. Similarly, heterozygous desmin null mice are also normal, while homozygous null mice develop multi-systemic abnormalities in all muscles, especially cardiac tissue [90]. These knockout data again suggest that both disorders would be amenable to disease allele-specific silencing.

LGMD1F-H: Therapeutic development for these disorders still awaits disease gene identification.

CONCLUSION AND FUTURE PERSPECTIVES

Over the last two decades, the feasibility of using gene therapy to treat muscular dystrophy in general, and LGMD in particular, has improved due to important technical advancements made in vector design, production and delivery. To date, both preclinical translational research in LGMD animal models and clinical trials in human patients have shown promising potential using gene therapy to ameliorate the histopathological and functional features of LGMD. With the progress of our knowledge in the diagnosis and mechanisms of LGMD as well as the availability of animal models, it is likely that gene therapy will become a major therapeutic approach for treating LGMD in the clinic.

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Table 1

Current Engineered Mouse Models with LMNA Mutations

Reference	LMNA mutation	Model intended for:	Actual phenotype hetero- or hemizygous	Homozygous
Sullivan, <i>et al.</i> 1999; Wolf, <i>et al.</i> 2008; Cupesi, <i>et al.</i> 2010	Null exon 8–11	AR-EDMD	No obvious skeletal myopathy; mild cardiac conduction defects after 10 wks; dilated cardiomyopathy at 50 wks	EDMD; death by 8 wks
Mounkes, <i>et al.</i> 2003	L530P knockin	AD-EDMD	Normal	Progeria syndrome; death by 4–5 wks
Mounkes, <i>et al.</i> 2005	N195K knockin	AD-CMD1A	Normal	CMD1A; death by 12–16 wks
Arimura, <i>et al.</i> 2005	H222P knockin	AD-EDMD	Normal	EDMD w dilated cardiomyopathy; death by 13 mos
Yang, <i>et al.</i> , 2005, 2008	intron 10-exon 11 knockin; produces uncleavable prelamin A called progerin	AD-HGPS	Progeria; death by 21–36 wks	Progeria; death by 4–17 wks
Varga, <i>et al.</i> 2006	Progerin G608G BAC transgenic	AD-HGPS	Progeria-related cardiovascular disease; no early death reported	NA
Wang, <i>et al.</i> 2006	M371K transgenic using α -MHC promoter for cardiac expression	AD-EDMD-associated cardiomyopathy	AD-EDMD-associated cardiomyopathy; death by 2–7 wks	NA
Wang, <i>et al.</i> 2008	Progerin G608G transgenic using K14 epidermal keratinocyte promoter	AD-HGPS skin abnormalities	Defects in keratinocyte nuclear structure but no skin abnormalities; no early death reported	NA
Sagelius, <i>et al.</i> 2008a, 2008b	Progerin G608G transgenic using inducible K5 epidermal keratinocyte promoter	AD-HGPS skin abnormalities	HGPS-associated skin abnormalities; death by 7–14 wks	NA
Wojtanik, <i>et al.</i> 2009	R482Q transgenic using α P2 adipose-specific promoter	AD-FPLD2	FPLD2-like; no early death reported	NA
Lu, <i>et al.</i> 2010	E82K transgenic using α -MHC promoter for cardiac expression	AD-CMD1A	CMD1A; death by 3–10 mos	NA

Table 2

Current Engineered Mouse Models with CAV3 Mutations

Reference	CAV3 mutation	Model intended for:	Actual phenotype hetero- or hemizygous	Homozygous
Hagiwara, <i>et al.</i> 2000; Woodman, <i>et al.</i> 2002	Null	AR-caveolinopathy	Normal	Skeletal myopathy after 8 wks; progressive cardiomyopathy beginning at 8 wks
Galbiati, <i>et al.</i> 2000; Aravamuden, <i>et al.</i> 2003	CAV3 over-expression using CAG promoter	Model of CAV3 over-expression seen in DMD and <i>mdx</i> mice	Skeletal myopathy by 3–4 wks; cardiomyopathy by 6–10 wks; death by 10–12 months	NA
Tsutsumi, <i>et al.</i> 2008; Horikawa, <i>et al.</i> 2011	CAV3 over-expression using α -MHC promoter to drive cardiomyocyte expression	Examining effects of CAV3 on natriuretic peptide signaling	Normal to beneficial	NA
Sunada, <i>et al.</i> 2001; Ohsawa, <i>et al.</i> 2004	P104L transgenic using MCK promoter	LGMD1C	Skeletal myopathy by 6 wks; hindlimb paralysis by 12 wks; hypertrophic cardiomyopathy by 6 wks	NA