Published in final edited form as:

Exp Physiol. 2014 April ; 99(4): 621–626. doi:10.1113/expphysiol.2013.075887.

Modifiers of Heart and Muscle Function: Where Genetics Meets Physiology

Kayleigh A. Swaggart¹ and Elizabeth M. McNally^{1,2}

¹Department of Human Genetics, The University of Chicago, Chicago, IL 60637

²Department of Medicine, The University of Chicago, Chicago, IL 60637

Abstract

Many single gene disorders are associated with a range of symptoms that cannot be solely explained by the primary genetic mutation. Muscular dystrophy is a genetic disorder associated with variable outcomes that arises from both the primary genetic mutation and the contribution from environmental and genetic modifiers. Disruption of the dystrophin complex occurs in Duchenne muscular dystrophy and limb girdle muscular dystrophy producing heart and muscle disease through a cellular injury process characterized by plasma membrane disruption and fibrosis. Multiple modifier loci have been mapped by using a mouse model of muscular dystrophy. These modifiers exert their effect often on specific muscle groups targeted by the muscular dystrophy process, possibly reflecting distinct pathophysiological processes among muscle groups. Genetic modifiers act on both cardiac and respiratory muscle parameters suggesting genetic and physiological integration of cardiopulmonary function. Skeletal muscles of the limbs are modified by a locus on mouse chromosome 7. This region of chromosome 7 harbors an insertion/deletion polymorphism in *Ltbp4*, the gene encoding latent TGF β binding protein. *Ltbp4* exerts its effect in muscle disease by acting on plasma membrane stability and fibrosis, thereby linking instability of the sarcolemma directly to fibrosis. In the human muscle disease Duchenne Muscular Dystrophy, protein coding single nucleotide polymorphisms in *LTBP4* associate with prolonged ambulation demonstrating that modifiers identified from mouse studies translate to human disease.

Keywords

muscular dystrophy; genetic modifiers; cardiopulmonary function

Modifiers of single gene disorders

The muscular dystrophies are a genetically heterogeneous group of single gene disorders marked by progressive muscle replacement and weakness. Although more than 40 genes have been linked to muscular dystrophy (MD) as primary gene mutations, a subgroup of genes share a pathological mechanism by disrupting the dystrophin complex. Dystrophin is a cytoskeletal protein that participates in a mechanically strong linkage that stabilizes the membrane, particularly during muscle contraction (Figure 1). When components of the dystrophin complex are compromised, the muscle membrane is susceptible to excessive tearing. Although regenerative mechanisms work to repair muscle fibers, the excessive damage caused by MD overwhelms the regenerative capacity. Sustained damage of the muscle fibers triggers degeneration and replacement of the fiber by fibrotic scar tissue. The

reduction in functional contractile fibers within the muscle causes progressive weakness, a hallmark clinical finding in MD.

The most common form of MD is Duchenne Muscular Dystrophy (DMD), caused by mutations in the dystrophin gene (*DMD*). In limb girdle muscular dystrophy type 2C (LGMD2C), the gene encoding the dystrophin-associated protein γ -sarcoglycan (*SGCG*) is disrupted. Both *DMD* and *SGCG* encode components of the dystrophin-glycoprotein complex, and loss of function mutations in either of these genes leads to severe muscle weakness and dilated cardiomyopathy (Figure 1). Becker Muscular Dystrophy (BMD) is milder than DMD because the BMD mutations are those that only partially disrupt the dystrophin gene. The enormous size of the dystrophin gene is associated with many unique mutations responsible for muscle disease, and mutations that cause a complete loss of dystrophin protein produce more severe disease, albeit with variability. Approximately half of patients with *SGCG* mutations have the same frameshifting mutation (McNally *et al.*, 1996). Despite this single mutation, the phenotypic outcome associated with this mutation varies considerably, affecting age at which patients lose ambulation and severity of cardiopulmonary involvement (McNally *et al.*, 1996; Kefi *et al.*, 2003). In the *Sgcg* mouse model of LGMD, which closely mimics the symptoms seen in humans, variability in disease severity is observed when the mutation is bred into different inbred strains of mice (Heydemann *et al.*, 2005). These findings in humans and mice demonstrate the existence of genetic modifiers of MD.

Genetic modifiers act in concert with the primary disease mutation to lessen or suppress disease severity, or to enhance it. While modifiers may have little phenotype in the absence of the primary mutation, they can significantly influence the symptoms, severity and outcome of disease. Genetic modifiers arise from the normal genomic variation that exists between humans. In mice, genetic modifiers can be mapped using inbred strains of mice because of the variation between different strains. Genetic variation, in either humans or mice, can alter protein coding regions to change the way the modifier gene functions or is processed. Genetic variation in noncoding regions may change when and where it is expressed.

Quantitative trait loci mapping to identify modifiers of MD

Determining the genetic architecture behind the variable phenotypes observed in MD is important for understanding the mechanisms of muscle and its dysfunction in disease. Mouse models of MD have been successfully used to identify modifier loci with quantitative trait locus (QTL) mapping. QTL mapping takes advantage of genetic and phenotypic variability and utilizes this information to detect regions of the genome that influence the trait being analyzed. To detect modifiers of MD, a genetically and phenotypically diverse cohort was created by crossing mice with MD (*Sgcg* mice) from the severely affected DBA/2J and mildly affected 129T2/SvEmsJ strains (referred to as *Sgcg*^{D2/129}). With each generation, the genomes of the progeny become more diverse with increasingly unique contributions from each parental strain (Figure 2). This mixing of genomes creates diversity in any phenotype with a genetic component. A number of methods were used to measure disease severity in the *Sgcg*^{D2/129} animals. Injection of Evans blue dye was used to quantify membrane damage in muscles. This dye binds to albumin and cannot permeate intact cells. However, when the sarcolemma is damaged, the dye enters the muscle fibers and dye content is a measure of membrane leakiness (Figure 2). Hydroxyproline, a modified amino acid found in collagen, is used to measure fibrosis in muscles, including the heart (Figure 2).

Intercrossed *Sgcg* mice from the severe and mild strains were used to detect modifiers of muscle membrane leak and fibrosis. Modifiers were found for limb based skeletal muscles

as well as the heart, diaphragm and abdominal muscles (Swaggart et al., 2011). A single locus on chromosome 3 regulated severity in the diaphragm and abdominal muscles (Figure 3). This region contains candidate genes of interest including the gene encoding netrin G1, and netrin pathways were previously suggested to interact with the dystrophin complex in a *Drosophila* screen (Kucherenko et al., 2008). The severity of disease in these two muscles is highly correlated. This, along with the finding that the same region regulated both abdominal and diaphragm muscle scarring, may reflect the recruitment of abdominal muscles as important respiratory muscles in MD, particularly when the diaphragm is significantly affected. In this study, a distinct region on chromosome 9 was found to associate with cardiac fibrosis. Cardiopulmonary function is significantly compromised in MD. Like in skeletal muscle, the heart and respiratory muscles are variably affected by the dystrophic process, becoming progressively replaced by fibrosis and becoming functionally impaired.

Protective modifiers in MD

The MRL/MpJ mouse strain is described as “superhealing” due to the ability to quickly repair acute injury such as that from an ear punch (Clark et al., 1998; McBrearty et al., 1998). To study the effects of the MRL genome in a chronic disease model like MD and to identify additional modifiers of MD, a cohort of *Sgcg* mice on a mixed MRL/DBA2J background was created (referred to as *Sgcg*^{D2/MRL}) (Figure 2). Similar measures of membrane leakiness and fibrosis were used and in addition, echocardiography was performed to assess cardiac function. It was found that a random contribution of the MRL genome was sufficient to improve MD (Heydemann et al., 2012). At eight weeks of age, a relatively early timepoint of disease in the mouse, fibrosis was significantly decreased in the abdominal, diaphragm, heart and quadriceps muscles of *Sgcg*^{MRL/D2}. This protection persisted in older mice (32 weeks of age) indicating that the MRL background can exert a sustained effect. The MRL genome partially reversed the cardiac dysfunction normally seen in *Sgcg* mice. The improved phenotype contributed by the MRL genome was not due to a general effect of crossing the severe DBA/2J strain to any more mildly affected strain. The 129T2/SvEmsJ genome was previously shown to protect against severe disease pathology in *Sgcg* mice, but its influence is less significant than that of MRL/MpJ. When compared to *Sgcg*^{D2/129} mice, *Sgcg*^{D2/MRL} mice showed significantly reduced fibrosis in all muscles examined. These improvements in pathology and function are thought to be due to genetic variation that enhances regeneration in MRL mice.

To identify the genetic drivers behind the improved muscle pathology and function seen in *Sgcg*^{D2/MRL} mice as a model of chronic injury, QTL mapping was performed (Heydemann et al., 2012). A single region on chromosome 2 was identified as a potential modifier of fibrosis in the heart, diaphragm and abdominal muscles (Figure 3). Given the tissues affected by the QTL, this finding suggests a broader role of the chromosome 2 locus in cardiopulmonary function. Taken together with the specificity of modifiers identified in the studies of *Sgcg*^{D2/129} mice, it should be possible to identify genes that are specifically responsible for the cardiopulmonary dysfunction seen in MD.

Linking genetics and cardiopulmonary function

Cardiopulmonary dysfunction in MD is clinically highly relevant. A number of QTLs affecting the heart and trunk based muscles of the *Sgcg* mouse model are of special interest due to their potential involvement in cardiopulmonary function. It is known that the diaphragm is consistently and extensively damaged in MD, leading to impaired respiratory function. The role of the abdominal muscles in MD and respiration is also a focus of attention. In humans, phenotypic variability in pulmonary function has been observed in

individuals with DMD, BMD, LGMD and Facio-Scapulo Humeral MD (Birnkrant et al., 2010; D'angelo et al., 2011), suggesting the presence of genetic modifiers for this aspect of disease. In addition, the contribution of the abdominal muscles to breathing has been linked to measures of respiratory performance. In DMD, reduced use of the abdominal muscles during breathing associated with decreased nighttime oxygen saturation (Romei et al., 2012). It is suggested that measurements of abdominal contribution could be an early indicator for the need for nighttime respiratory support. If so, identifying any genetic drivers behind this phenotype will be important for predicting outcome and developing treatment plans. The abdominal muscle modifier loci identified in mice may also be important for overall respiratory function in humans with MD.

Reduced respiration and the resulting hypoxia may trigger pulmonary vasoconstriction and increased pulmonary artery pressure. This increase is expected to increase workload for the right ventricle, further exacerbating the contraction-induced damage typical of MD. The role of respiration in overall cardiopulmonary function makes it an important target for study and treatment. In work using the *mdx/utrn* double null mouse model of DMD, it was shown that rescue of diaphragm function through upregulation of utrophin, a gene that can functionally compensate in the absence of dystrophin, is sufficient to restore heart function (Crisp et al., 2011). It can be hypothesized that rescue of abdominal muscle function may have the same effect, making genetic modifiers specifically affecting these muscles obvious candidates for study and targeted treatment.

Modifiers of limb muscles in MD

Ltbp4 was previously found to be a modifier of limb skeletal muscle severity in the *Sgcg* mouse model of MD (Heydemann et al., 2009). The chromosome 7 region where the *Ltbp4* gene is found was highly significantly linked to sarcolemmal damage and fibrosis in the quadriceps muscles (Figure 3). In the murine *Ltbp4* gene, there is a protein altering 36 base pair deletion found in the DBA/2J strain. This 36 base deletion removes 12 amino acids from the proline rich hinge region of LTBP4, which alters its ability to bind and sequester TGF β (Heydemann et al., 2009). LTBP4s and TGF β are secreted together forming the large latent complex, and the interaction between LTBP4s and TGF β is important for the intracellular secretion process as well as matrix deposition (Todorovic & Rifkin, 2012). TGF β is an important regulator of injury and fibrosis (Ghosh et al., 2013). The insertion/deletion polymorphism in murine *Ltbp4* alters the proteolytic susceptibility of the LTBP4 protein and is directly correlated with altered TGF β signaling (Heydemann et al., 2009). In the severe DBA/2J strain, LTBP4 is more readily cleaved, resulting in more TGF β signaling and this is seen as increased fibrosis in *Sgcg* DBA/2J mice. Alone, this variation in *Ltbp4* causes no obvious phenotype. However, when in the context of MD, it has a profound affect on how muscle fibers respond to damage.

Natural variation also occurs in the human *LTBP4* gene. While not an insertion/deletion polymorphism like that observed in mice, there are four nonsynonymous variants that form two common haplotypes in the general population (Flanigan et al., 2012). Because these two haplotypes are well represented in the general population, most individuals are heterozygous carrying one each of the two major haplotypes. A smaller percentage of the population (~15%) is homozygous for one or the other haplotype of *LTBP4*. In DMD, boys homozygous for the protective *LTBP4* haplotype walk significantly longer than those heterozygous or homozygous for the deleterious haplotype (Flanigan et al., 2012). This effect is magnified by steroid treatment. DMD patients treated with steroids and homozygous for the protective allele showed significantly prolonged ambulation, while those who did not take steroids had a nonsignificant association. These data suggest that LTBP4 may act by modifying inflammation in concert with steroid use. The gene-drug-

disease interaction demonstrates the role of genetic and environmental modifiers in determining clinical outcome. These findings suggest that genetic modifiers can have a general role in multiple forms of MD and illustrate the importance of these loci on skeletal muscle pathology and function in single gene disorders.

Conclusions

Mouse models are an important tool in the study of the genetics and physiology of MD. In addition to mimicking the phenotypes and variability observed in humans, these animals have been successfully used to map modifiers that also affect human disease. The modifiers mapped in mice support common modifiers for the diaphragm and abdominal muscles, likely arising from the use of abdominal muscles as respiratory muscles, a suggestion supported by human studies. In at least one case, the same modifier that regulates respiratory muscle function also links to cardiac muscle fibrosis consistent with global cardiopulmonary modifiers. Identification of these modifiers may help predict cardiopulmonary outcomes in MD. A clearer picture of the genetic architecture behind MD will also assist in identifying targets for treatment.

Acknowledgments

Supported by NIH HL61322, AR052646, NS072027.

References

- Birnkrant DJ, Ashwath ML, Noritz GH, Merrill MC, Shah TA, Crowe CA, Bahler RC. Cardiac and pulmonary function variability in Duchenne/Becker muscular dystrophy: an initial report. *J Child Neurol.* 2010; 25:1110–1115. [PubMed: 20501885]
- Clark LD, Clark RK, Heber-Katz E. A new murine model for mammalian wound repair and regeneration. *Clin Immunol Immunopathol.* 1998; 88:35–45. [PubMed: 9683548]
- Crisp A, Yin H, Goyenvalle A, Betts C, Moulton HM, Seow Y, Babbs A, Merritt T, Saleh AF, Gait MJ, Stuckey DJ, Clarke K, Davies KE, Wood MJ. Diaphragm rescue alone prevents heart dysfunction in dystrophic mice. *Hum Mol Genet.* 2011; 20:413–421. [PubMed: 21062902]
- D'angelo MG, Romei M, Lo Mauro A, Marchi E, Gandossini S, Bonato S, Comi GP, Magri F, Turconi AC, Pedotti A, Bresolin N, Aliverti A. Respiratory pattern in an adult population of dystrophic patients. *Journal of the Neurological Sciences.* 2011; 306:54–61. [PubMed: 21529845]
- Flanigan KM, Ceko E, Lamar KM, Kaminoh Y, Dunn DM, Mendell JR, King WM, Pestronk A, Florence JM, Mathews KD, Finkel RS, Swoboda KJ, Gappmaier E, Howard MT, Day JW, McDonald C, McNally EM, Weiss RB. LTBP4 genotype predicts age of ambulatory loss in duchenne muscular dystrophy. *Ann Neurol.* 2012 in press.
- Ghosh AK, Quaggin SE, Vaughan DE. Molecular basis of organ fibrosis: Potential therapeutic approaches. *Exp Biol Med (Maywood).* 2013; 238:461–481. [PubMed: 23856899]
- Heydemann A, Ceko E, Lim JE, Hadhazy M, Ryder P, Moran JL, Beier DR, Palmer AA, McNally EM. Latent TGF-beta-binding protein 4 modifies muscular dystrophy in mice. *J Clin Invest.* 2009; 119:3703–3712. [PubMed: 19884661]
- Heydemann A, Huber JM, Demonbreun A, Hadhazy M, McNally EM. Genetic background influences muscular dystrophy. *Neuromuscul Disord.* 2005; 15:601–609. [PubMed: 16084087]
- Heydemann A, Swaggart KA, Kim GH, Holley-Cuthrell J, Hadhazy M, McNally EM. The superhealing MRL background improves muscular dystrophy. *Skelet Muscle.* 2012; 2:26. [PubMed: 23216833]
- Kefi M, Amouri R, Driss A, Ben Hamida C, Ben Hamida M, Kunkel LM, Hentati F. Phenotype and sarcoglycan expression in Tunisian LGMD 2C patients sharing the same del521-T mutation. *Neuromuscul Disord.* 2003; 13:779–787. [PubMed: 14678800]

- Kucherenko MM, Pantoja M, Yatsenko AS, Shcherbata HR, Fischer KA, Maksymiv DV, Chernyk YI, Ruohola-Baker H. Genetic modifier screens reveal new components that interact with the *Drosophila* dystroglycan-dystrophin complex. *PLoS One*. 2008; 3:e2418. [PubMed: 18545683]
- McBrearty BA, Clark LD, Zhang XM, Blankenhorn EP, Heber-Katz E. Genetic analysis of a mammalian wound-healing trait. *Proc Natl Acad Sci U S A*. 1998; 95:11792–11797. [PubMed: 9751744]
- McNally EM, Passos-Bueno MR, Bonnemann CG, Vainzof M, de Sa Moreira E, Lidov HG, Othmane KB, Denton PH, Vance JM, Zatz M, Kunkel LM. Mild and severe muscular dystrophy caused by a single gamma-sarcoglycan mutation. *Am J Hum Genet*. 1996; 59:1040–1047. [PubMed: 8900232]
- Romei M, D'Angelo MG, LoMauro A, Gandossini S, Bonato S, Brighina E, Marchi E, Comi GP, Turconi AC, Pedotti A, Bresolin N, Aliverti A. Low abdominal contribution to breathing as daytime predictor of nocturnal desaturation in adolescents and young adults with Duchenne Muscular Dystrophy. *Respir Med*. 2012; 106:276–283. [PubMed: 22083092]
- Swaggart KA, Heydemann A, Palmer AA, McNally EM. Distinct genetic regions modify specific muscle groups in muscular dystrophy. *Physiol Genomics*. 2011; 43:24–31. [PubMed: 20959497]
- Todorovic V, Rifkin DB. LTBP5, more than just an escort service. *J Cell Biochem*. 2012; 113:410–418. [PubMed: 22223425]

Abbreviations

BMD	Becker Muscular Dystrophy
DMD	Duchenne Muscular Dystrophy
LGMD	Limb Girdle Muscular Dystrophy
<i>Ltbp4</i>	latent TGF β binding protein
MD	Muscular Dystrophy
QTL	Quantitative Trait Locus
TGFβ	transforming growth factor-beta

New Findings

What is the topic of the review?

Genetic modifiers act on many different physiological aspects of muscle disease. Understanding and identifying such modifiers is important because their discovery may help predict the course of muscle disease and also indicate pathways to be exploited in designing new therapeutics.

What advances does it highlight?

Genetic modifiers have been identified that act primarily on limb skeletal muscles. Newer modifiers, where the responsible gene has yet to be identified, alter the course of cardiopulmonary dysfunction in muscular dystrophy. Distinct modifiers that act differentially on limb skeletal versus heart and respiratory muscles reflect underlying physiological differences of these muscle groups.

Figure 1.

The dystrophin-glycoprotein complex is disrupted in many forms of muscular dystrophy. The complex serves to link the extracellular matrix to the actin cytoskeleton within the muscle fiber. When components of the dystrophin complex are compromised, the complex and sarcolemma are destabilized. Dystrophin is a cytoplasmic protein that links the actin cytoskeleton to the complex (pink). Mutations in dystrophin that lead to a complete loss of protein result in Duchenne Muscular Dystrophy (DMD). Those mutations that preserve some dystrophin protein expression lead to the milder Becker Muscular Dystrophy (BMD). The sarcoglycan complex within the dystrophin-glycoprotein complex consists of four proteins: α -, β -, γ - and δ -sarcoglycan (green). Mutations in these genes lead to the Limb Girdle Muscular Dystrophies (LGMDs). In LGMD type 2C, γ -sarcoglycan is disrupted.

Figure 2.

Mapping modifiers in mice requires a genetically and phenotypically diverse cohort. To create variability, *Sgcg* animals lacking γ -sarcoglycan from the severely affected inbred mouse strain DBA/2J were crossed with the mildly affected 129T2 strain (A). The progeny of the initial cross were interbred, creating the F2 cohort used for analysis. To study the effect of the superhealing MRL/MpJ genome on disease and identify modifiers of their regenerative phenotype, mice from this strain were crossed with *Sgcg* mice on the DBA/2J background (B). In this second cohort, animals were intercrossed to the F4 generation. F2, F3 and F4 mice were used for modifier mapping. Animals from each cross were analyzed for membrane damage and fibrosis. Evans blue dye (dye) was used to mark damaged muscle fibers. Image C shows skeletal muscle in cross section. Fibers are outlined with dystrophin (green); dye positive fibers autofluoresce red, which appears orange because of double staining with green dystrophin. Fibrosis was quantified via hydroxyproline content, a modified amino acid found in collagen. Using Masson's trichrome staining, fibrotic infiltrate appears blue, while intact muscle fibers appear red (D).

Figure 3.

A region on chromosome 2 was found to modify fibrosis in the heart, diaphragm and abdominal muscles (purple) in mice using the *Sgcg*^{D2/MRL} mapping cohort. Using the *Sgcg*^{D2/129} mapping cohort, a region on mouse chromosome 3 was identified as a modifier of fibrosis in the diaphragm and abdominal muscles (blue). A region on mouse chromosome 9 modified cardiac fibrosis (pink). This study also identified a region of mouse chromosome 7 that modifies multiple limb-based skeletal muscles (green). Phenotypes affected by this QTL include fibrosis and membrane damage in the quadriceps muscle, as well as membrane damage in the gastrocnemius/soleus, gluteus/hamstring and triceps muscles. In mice, the chromosome 7 QTL includes the gene *Ltbp4*. In humans, common genetic variation in *LTBP4*, found on chromosome 19, correlates significantly with prolonged ambulation in boys with DMD.