

Gene Therapy in Large Animal Models of Human Cardiovascular Genetic Disease

Meg M. Sleeper, Lawrence T. Bish, and H. Lee Sweeney

Abstract

Several naturally occurring animal models for human genetic heart diseases offer an excellent opportunity to evaluate potential novel therapies, including gene therapy. Some of these diseases—especially those that result in a structural defect during development (e.g., patent ductus arteriosus, pulmonic stenosis)—would likely be difficult to treat with a therapeutic gene transfer approach. However, the ability to transduce a significant proportion of the myocardial cells should make the various forms of inherited cardiomyopathy amenable to a therapeutic gene transfer approach. Adeno-associated virus may be the ideal vector for cardiac gene therapy since its low immunogenicity allows for stable transgene expression, a crucial factor when considering treatment of a chronic disease. Cardiomyopathies are a major cause of morbidity and mortality in both children and adults, and large animal models are available for the major forms of inherited cardiomyopathy (dilated cardiomyopathy, hypertrophic cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy). One of these animal models, juvenile dilated cardiomyopathy of Portuguese water dogs, offers an effective means to assess the efficacy of therapeutic gene transfer to alter the course of cardiomyopathy and heart failure. Correction of the abnormal metabolic processes that occur with heart failure (e.g., calcium metabolism, apoptosis) could normalize diseased myocardial function. Gene therapy may offer a promising new approach for the treatment of cardiac disease in both veterinary and human clinical settings.

Key Words: calcium metabolism; cardiomyopathy; dog model; gene transfer; heart disease; heart failure; large animal model

Introduction

Naturally occurring animal models (e.g., beagles, keeshonds, and Newfoundland dogs) exist for many inherited cardiovascular diseases in humans (Patterson 1978; Patterson and Detweiler 1967; Patterson et al. 1974, 1981, 1982, 1993; Pyle et al. 1976). In fact, re-

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searchers have used several of these models in breeding colonies to describe the natural history of the disease and study inheritance patterns (Van Mierop and Patterson 1978; Van Mierop et al. 1977; Werner et al. 1999, 2005). However, to our knowledge, none of these models of congenital cardiac defects is being used to investigate the potential for gene therapy in genetic cardiovascular diseases. These diseases would likely be difficult to treat with this approach since the defect occurs during development, but with the transduction of a significant proportion of the myocardial cells the various forms of inherited cardiomyopathy should be amenable to a therapeutic gene transfer approach.

Cardiomyopathies are diseases of the myocardium that cause systolic and/or diastolic dysfunction and may result in heart failure, cardiac arrhythmias, and sudden death. They are a major cause of morbidity and mortality in both children and adults. In 1995, the World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies recommended updating its classification system to better reflect the current understanding of the etiology and pathogenesis of cardiomyopathies, which are now classified by dominant pathophysiology (Richardson et al. 1996).

Dilated cardiomyopathy may be idiopathic, familial/genetic, viral and/or immune, or toxic, whereas hypertrophic cardiomyopathy and arrhythmogenic right ventricular cardiomyopathy are frequently familial (Richardson et al. 1996). The phenotype for inherited cardiomyopathies is often not expressed until the individual is an adult. Some genetic heart diseases, such as Duchenne muscular dystrophy, also affect skeletal muscle; however, in this review we focus on those that affect only cardiac muscle, and refer interested readers to the article in this issue that discusses Duchenne muscular dystrophy (Wang et al. 2009).

Human Cardiac Genetic Diseases That Naturally Occur in Large Animal Models

Dilated Cardiomyopathy

Dilated Cardiomyopathy in Humans

Idiopathic dilated cardiomyopathy (DCM¹) causes cardiac chamber dilation, ventricular wall thinning, and reduced

¹Abbreviations used in this article: AAV, adeno-associated virus; ARVD, arrhythmogenic right ventricular dysplasia; DCM, dilated cardiomyopathy;

ventricular contractility and is the most common cause of congestive heart failure in the young (less than 18 years of age), with an estimated prevalence in the United States of at least 36.5 per 100,000 persons (Hughes and McKenna 2005). The cumulative survival rates vary—75-80% at 1 year, and 60-75% at 5 years (Azevedo et al. 2007)—and the incidence of heart failure doubles each decade of life beginning in the fifth (Ho et al. 1993). Although the etiology is often unknown, up to 35% of individuals with idiopathic DCM have familial disease (Hughes and McKenna 2005); the pattern of inheritance is variable and includes autosomal dominant, X-linked, autosomal recessive, and mitochondrial inheritance (Hughes and McKenna 2005). In familial forms, the mutated genes are most often those that encode for cytoskeletal proteins related to force transmission such as cardiac actin or desmin (Thiene et al. 2005).

Dilated Cardiomyopathy in Animals

Researchers in the 1990s recognized an inherited form of DCM in juvenile Portuguese water dogs (PWDs¹) (Dambach et al. 1999). The disease, inherited as an autosomal recessive trait, is rapidly progressive, with an average age of death from congestive heart failure at 13 weeks. Affected puppies progress from a normal echocardiographic appearance to severe left ventricular dilation and systolic failure within 1 to 4 weeks of the first echocardiographic alterations (Sleeper et al. 2002). A genomewide linkage study to identify the locus responsible for the disease did not determine the specific gene, but revealed that the locus maps to a 3.9 Mb region on canine chromosome 8, which is homologous to human chromosome 14 (Werner et al. 2008). There is no evidence that genes or loci from this region are involved in the development of human cardiomyopathy, so this discovery will provide a new gene to examine in human DCM patients.

For these reasons, juvenile DCM-affected PWDs are useful models for testing therapeutic approaches to heart failure (Werner et al. 2008). A colony of these dogs has been established at the University of Pennsylvania School of Veterinary Medicine under National Institutes of Health and US Department of Agriculture guidelines for the care and use of animals in research (NRC 1996). Carrier dogs are being bred to support research that will identify the causative mutation and to test novel therapeutic approaches, such as gene transfer. Although other breeds of dogs develop adult onset DCM with increased prevalence and a genetic basis has been suggested, the molecular etiology remains unresolved (Meurs et al. 2001a,b).

Hypertrophic Cardiomyopathy

Hypertrophic Cardiomyopathy in Humans

Hypertrophic cardiomyopathy (HCM¹) is characterized by left ventricular hypertrophy, myocyte disarray, and diastolic

dysfunction. In a subset of patients, left ventricular outflow tract obstruction causes a cardiac murmur; however, physical examination is often unreliable because many affected patients do not have left ventricular outflow tract obstruction or any other abnormalities causing a murmur that would be detectable in a physical examination (Marron 2002). Therefore, 2-dimensional echocardiography is the easiest and most reliable diagnostic tool for identifying affected individuals (Marron 2002). Unexplained hypertrophy occurs in 1:500 in the general population, making hypertrophic cardiomyopathy, inherited as a Mendelian autosomal dominant trait (Marron 2002), the most common inherited cardiac disorder (Sherrid 2006).

The HCM phenotype is not static; for example, left ventricular hypertrophy can appear at virtually any age and increase or decrease dynamically throughout life. It is therefore impossible to use a normal echocardiogram to offer definitive reassurance at maturity that asymptomatic family members are free of a disease-causing mutant HCM gene (Marron 2002). The disease has been associated with mutations of 10 genes that code for myofilaments or their supporting proteins, although the most common mutations are present in the β -myosin heavy chain and in myosin-binding protein C (Sherrid 2006).

Hypertrophic Cardiomyopathy in Animals

A naturally occurring feline model of familial HCM, inherited in an autosomal dominant pattern, was described in 1999 in a family of Maine coon cats (Kittleson et al. 1999). Following the development of a colony of these cats at the University of California at Davis, researchers have described the phenotypic expression and natural history of disease (MacDonald et al. 2006, 2007). The causative mutation is a single base pair change that results in a conformational change of the sarcomeric protein, myosin-binding protein C. As with HCM-affected humans, the cats show phenotypic variation in the severity of the disease (Meurs et al. 2005). More recently, the same group reported that a separate mutation in the myosin-binding protein C gene causes HCM in a different breed of cat, the Ragdoll (also inherited in an autosomal dominant pattern) (Meurs et al. 2007b).

Arrhythmogenic Right Ventricular Dysplasia

Arrhythmogenic Right Ventricular Dysplasia in Humans

Arrhythmogenic right ventricular dysplasia (ARVD¹) is an inherited cardiomyopathy characterized by right ventricular dysfunction, fibro-fatty infiltration, and ventricular arrhythmias. The arrhythmias are most commonly induced by exercise and appear to be associated with significant remodeling of structures involved with cell-to-cell communication (Oxford et al. 2007). The estimated prevalence in the general population ranges from 1 in 2000 to 1 in 5000, and a familial background

HCM, hypertrophic cardiomyopathy; PLB, phospholamban; PWD, Portuguese water dog; SERCA, sarcoplasmic reticulum Ca²⁺ ATPase

is a factor in more than 50% of the cases (Corrado and Thiene 2006). More specifically, studies have shown that ARVD is present in up to 20% of individuals who experience sudden cardiac death, and appears to be even more common among athletes who die suddenly (Dalal et al. 2005). An autosomal dominant pattern of inheritance with incomplete penetrance is most common. ARVD is commonly referred to as a desmosomal disease, and multiple mutations in several genes involved in cell-to-cell junction (such as plakoglobin, desmoplakin, and plakophilin) have been implicated in its etiology; however, the disease has also been linked to genes coding for proteins that are not associated with the cell adhesion complex, including the ryanodine receptor and transforming growth factor- β 3 (Corrado and Thiene 2006).

Arrhythmogenic Right Ventricular Dysplasia in Animals

Basso and colleagues (2004) reported on a series of 23 boxer dogs, several of which were related, with clinical and pathologic signs very similar to ARVD. Familial transmission was suggested, but the precise pattern of inheritance was not clear; mutations in the desmosomal genes associated with the disease in humans were not present in the boxer dog (Meurs et al. 2007a). However, the message and protein expression of the ryanodine receptor were lower in all chambers of the ARVD-affected boxer dog compared to a normal dog, and differential expression was present in the normal dog (the right ventricular expression level was lower than the left), suggesting the disease may be associated with the ryanodine receptor in boxer dogs (Meurs et al. 2006). In spite of these results, the scientists did not identify linkage to the ryanodine receptor gene; however, they found the inheritance pattern to be consistent with an autosomal dominant pattern in the families studied (Meurs et al. 2006). A recent study validated the boxer model of ARVD by demonstrating loss of gap junction plaques at the sites of cell apposition, which is similar to the abnormality seen in humans (Oxford et al. 2007). Studies have reported the disease in the domestic cat, but have not identified a familial pattern in this species (Fox et al. 2000; Harvey et al. 2005).

Gene Therapy Approaches

Background

Cardiovascular disease remains a leading cause of mortality worldwide (Cohn et al. 1997), and there are not enough healthy hearts available for all the patients waiting for a heart transplant—although approximately 60,000 cases per year could benefit from cardiac transplant, only about 2500 donor hearts are available (Bridges et al. 2002). Therefore novel therapeutic strategies are needed to augment the current treatment arsenal for heart failure.

Investigators are studying new approaches that target the underlying molecular defects of ventricular dysfunction.

Gene therapy is one molecular-based option for heart disease patients. It has traditionally been used to transfer a gene that encodes a functional protein into a diseased patient, to produce long-term expression of the deficient protein (Lyon et al. 2008; Vinge et al. 2008). This strategy, often referred to as gene replacement therapy, requires identification of the mutated gene. But gene transfer can also be effective when the causative mutation is unknown, with the goal of increasing the concentration of a therapeutic gene product in a tissue or organ. This use of gene transfer results in a “drug effect.” The approach can be effective whether or not the underlying genetic defect has been identified or the heart disease is genetic or acquired.

There is substantial evidence that impairment of Ca^{2+} handling is a final common stage in the pathophysiology of heart disease and failure (Manning et al. 2000; Most et al. 2004; Pleger et al. 2007). The intracellular calcium gradient is maintained by the cardiac sarcoplasmic reticulum Ca^{2+} ATPase (SERCA¹), which is modulated by several proteins including phospholamban (Dieterle et al. 2005). By altering the expression levels of the proteins that move calcium between the cytosol and the sarcoplasmic reticulum, it is possible to normalize Ca^{2+} handling in diseased myocardial cells, resulting in improved cardiac function regardless of the underlying disease. Moreover, using explanted cardiac specimens from human patients undergoing heart transplants, studies have shown that phospholamban expression increases in dilated cardiomyopathy although SERCA levels are not altered (Lennon et al. 2000). This change in phospholamban expression could explain the decreased Ca^{2+} uptake in diseased hearts. The reduced rate of Ca^{2+} removal into the sarcoplasmic reticulum results in impaired cardiac relaxation and a decrease in the amount of Ca^{2+} released via the ryanodine receptor. Therefore, elevated phospholamban levels appear to be a particularly significant factor in the disease process of dilated cardiomyopathy.

Many experiments with small animals have focused on altering levels of calcium-handling proteins by altering levels of various Ca^{2+} regulators such as phospholamban, β -adrenergic receptor kinase, and S100A1. Dieterle and colleagues (2005) used adenovirus to overexpress a recombinant, intracellularly expressed antibody-derived protein targeting the cytoplasmic domain of phospholamban (PLB¹) in a cardiomyopathic hamster model, and showed that short-term expression improved left ventricular function and myocardial contractility in the failing heart. Another group used a recombinant adeno-associated viral vector expressing a pseudophosphorylated mutant of PLB; the resultant gene product, acting as a dominant negative mutant, suppressed progressive impairment of left ventricular function and contractility for up to 30 weeks in the BIO 14.6 cardiomyopathic hamster, a model of limb-girdle muscular dystrophy type F, and the MLP-deficient cardiomyopathic mouse (Hoshijima et al. 2002).² Other groups have altered calcium cycling by

²Adenovirus-mediated delivery of this pseudophosphorylated PLB mutant was also effective in reversing heart failure progression in a sheep model of pacing-induced failure (Kaye et al. 2007).

targeting the β -adrenergic receptor (β AR) or its regulating kinase. For example, one group used a transgenic mouse model to show that acute β AR kinase inhibition can restore lost myocardial β AR responsiveness and adrenergic reserve (Manning et al. 2000). Furthermore, S100A1—a Ca^{2+} -sensing protein that increases myocardial SERCA activity, diminishes diastolic sarcoplasmic Ca^{2+} leakage, and results in an overall gain in sarcoplasmic reticulum Ca^{2+} cycling—has proven to have great potential as a therapeutic myocardial transgene; studies have shown that it improves myocardial function and reduces cardiac remodeling in a rat model of heart failure (Most et al. 2004; Pleger et al. 2007).

Increased rates of apoptosis, or programmed cell death, have also been demonstrated in humans and animal models with various forms of heart disease (Olivetti et al. 1997). Gene therapy using an antiapoptotic factor (Bcl-2) was protective in a rabbit model of ischemic heart disease, conferring protection from apoptosis throughout the 6 weeks of the study and resulting in preserved left ventricular geometry and prevention of dilation (Chatterjee et al. 2002). Gene transfer of ARC, the apoptosis repressor with a caspase-recruiting domain, had similar efficacy in the same rabbit model of heart failure (Chatterjee et al. 2003).

Cardiac Gene Transfer

As suggested above, cardiac gene therapy is simple in the small animal, as multiple studies have demonstrated stable and efficient global myocardial transgene expression in rodent models using adeno-associated viral vector (Gregorevic et al. 2004; Inagaki et al. 2006; Woo et al. 2005). Adeno-associated virus (AAV¹) is an ideal cardiac gene transfer vector because its low immunogenicity favors persistent transgene expression. Although at least one study suggests that an immune response may reduce long-term expression of AAV-mediated gene transfer in humans (Manno et al. 2006), immune suppression appears to be effective (Wang et al. 2007). For a summary of the properties of the most commonly used gene transfer vectors, see Table 1.

Myocardial transduction has proven more difficult in large animals (Bridges et al. 2002, 2005), regardless of vector choice, but large animal models are nonetheless likely to be superior to small animal models for several reasons. Although mice have the advantage of being easy to breed, thus providing large numbers of affected animals for evaluation, they have clearly defined and uniform genetic backgrounds. Larger species such as the dog and cat, on the other hand, have a heterogeneous genetic background and are of a size more suited for surgical manipulations and clinical evaluations. In addition, many large animal models of naturally occurring disease are homologues for human genetic diseases, the size of cats and dogs is useful for scaling to human trials, and their lifespans are more suited for long-term evaluation of possible gene therapy sequelae (Ponder and Haskins 2007). Finally, the hearts of larger animals are molecularly and physiologically more similar to human hearts than are those of rodents: the β -myosin heavy chain is the predominant isoform in adults, cytosolic calcium removal is performed 70% by SERCA and 30% by the $\text{Na}^{2+}/\text{Ca}^{2+}$ exchanger, and the force-frequency relationship is positive (Hasenfuss 1998).

Transduction Techniques

Groups have tried various methods to achieve global cardiac transduction in large animals. While it is unclear exactly what percentage of the myocardium needs to be successfully transduced for effective therapy, and the required number of transduced cells may vary depending on the underlying cause of cardiomyopathy, it is likely that at least 50% of the cells should be transduced. Bridges and colleagues (2005) demonstrated efficient (approximately 50%) global cardiac expression in a small group of mongrel dogs using β -galactosidase as a reporter transgene with a technique that entailed completely isolating the heart in situ (on cardiopulmonary bypass). Then, after retrograde infusion into the coronary sinus of 10^{13} particles of adenovirus encoding the reporter transgene in addition to 15 μg of vascular endothelial growth factor, they

Table 1 Properties of gene transfer vectors in the rabbit heart^a

	Positive cells/field	Stability of expression	Immune response ^b
Naked plasmid DNA	0	n.a.	No
Complexed ^c DNA	0	n.a.	No
Adenovirus	357	<21 days	Robust
Herpes simplex virus	16	<21 days	Robust
Adeno-associated virus	31	>21 days	No

n.a., not applicable.

^aFollowing direct intramyocardial injection (data from Wright MJ, Wightman LM, Lilley C, de Alwis M, Hart SL, Miller A, Coffin RS, Thrasher A, Latchman DS, Marber MS. 2001. In vivo myocardial gene transfer: Optimization, evaluation and direct comparison of gene transfer vectors. *Basic Res Cardiol* 96:227-236).

^bCompared to control (direct injection of vehicle only).

^cComplexed = plasmid DNA, cationic liposome, and integrin-targeting peptide

recirculated the mixture for 30 minutes at pressures ranging from 60 to 80 mm Hg (Bridges et al. 2005). Although the procedure resulted in efficient expression, one out of six normal dogs did not survive it, and it has not been reported in cardiomyopathic dogs. Another group demonstrated that infusion of adenovirus simultaneously through both the left anterior descending artery and great cardiac vein resulted in gene transfer to 78% of the perfused target area in swine (Sasano et al. 2007). Both studies used the highly immunogenic and unstable adenovirus vector.

We have developed a system involving the delivery of intramyocardial injections (40–60) of AAV throughout the left ventricle after percutaneous introduction of a cardiac injection catheter using a carotid artery approach. We believe this technique will be better tolerated by patients with heart disease than direct injections via thoracotomy or other more invasive techniques such as the procedure described above (Bridges et al. 2002, 2005). It also eliminates the requirement for costly and potentially dangerous vascular endothelial growth factor (VEGF), and, because it is independent of coronary perfusion, it should be effective for treating both ischemic and nonischemic heart disease. We have also demonstrated that self-complementary recombinant adeno-associated virus (rAAV) results in superior expression compared to single-stranded rAAV (Bish et al. 2008). Self-complementary AAV2/6 results in transduction of approximately 60% of the myocardium, which is on the order of 1 log superior to the expression obtained using self-complementary rAAV2/8 or rAAV2/9 (Bish et al. 2008). We are using this technique to inhibit phospholamban (via shRNA and dominant negative approaches) in the PWD model.

The Portuguese Water Dog (PWD) Model

Portuguese water dogs represent an excellent large animal model of dilated cardiomyopathy. Because the disease is extremely rapidly progressive, the lack of variability in affected dogs should help researchers recognize clinical changes secondary to gene transfer. Moreover, husbandry and housing costs are lower as affected dogs develop the disease at an early age. We are assessing techniques to suppress myocardial phospholamban expression with the hope that the results will be directly applicable to human cardiomyopathic patients.

In our first therapeutic attempt, we treated three PWDs with a scAAV6 vector expressing shRNA targeted against phospholamban using our catheter-based myocardial injection technique. After euthanizing the animals 2 to 4 weeks after treatment to assess PLB knockdown, we found a sixteenfold reduction in PLB mRNA and approximately a fivefold reduction in PLB protein compared to untreated control PWDs (Bish, Sleeper, Sweeney, unpublished data). Although these protein levels are approximately half those of healthy canines, we do not anticipate that this reduction will be detrimental since a cross of the phospholamban knockout (KO) mouse with the muscle LIM protein KO mouse (for a model of dilated cardiomyopathy) led to complete

correction of the cardiac phenotype (Minamisawa et al. 1999). Because knockdown was very effective, we are analyzing long-term safety and efficacy.

Other naturally occurring large animal models such as the ARVD boxer dog and the HCM Maine coon cat will also be useful as gene therapy approaches make the transition from small animals to human patients, but to the best of our knowledge gene therapy trials do not currently use these large animal models.

Summary

Therapeutic gene transfer holds great promise as a potential treatment for inherited cardiomyopathies, whether or not the causative gene is known, by normalizing the metabolic state of diseased myocardial cells. Because calcium-handling abnormalities (Diedrichs et al. 2007) and increased rates of apoptosis (Chatterjee et al. 2002; Olivetti et al. 1997) occur in many different types of heart disease, these approaches could be beneficial for dilated, hypertrophic, or arrhythmic right ventricular cardiomyopathy.

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References

- Azevedo VM, Santos MA, Filho FMA, Castier MB, Tura BR, Amino JGC. 2007. Outcome factors of idiopathic dilated cardiomyopathy in children: A long-term follow-up review. *Cardiol Young* 17:175-184.
- Basso C, Fox PR, Meurs KM, Towbin JA, Spier AW, Calabrese F, Maron BJ, Thiene G. 2004. Arrhythmogenic right ventricular cardiomyopathy causing sudden cardiac death in boxer dogs: A new animal model of human disease. *Circulation* 109:1180-1185.
- Bish LT, Sleeper MM, Brainard B, Cole S, Russell N, Withnall E, Arndt J, Reynolds C, Davison E, Sanmiquel J, Wu D, Gao G, Wilson JM, Sweeney HL. 2008. Percutaneous transendocardial delivery of self-complementary adeno-associated virus 6 achieves global cardiac gene transfer in canines. *Mol Ther* 16:1953-1959.
- Bridges CR, Burkman JM, Malekan R, Konig SM, Chen H, Yarnall CB, Gardner TJ, Stewart AS, Stecker MM, Patterson T, Stedman HH. 2002. Global cardiac-specific transgene expression using cardiopulmonary bypass with cardiac isolation. *Ann Thorac Surg* 73:1939-1946.
- Bridges CR, Gopal K, Holt DE, Yarnall C, Cole S, Anderson RB, Yin X, Nelson A, Kozyak BW, Wang Z, Lesniewski J, Su LT, Thesieur DM, Sunder H, Stedman HH. 2005. Efficient myocyte gene delivery with complete cardiac surgical isolation in situ. *J Thorac Cardiovasc Surg* 130:1364.
- Chatterjee S, Stewart AS, Bish LT, Jayasankar V, Kim EM, Pirolli T, Burdick J, Woo YJ, Gardner TJ, Sweeney HL. 2002. Viral gene transfer of the antiapoptotic factor Bcl-2 protects against chronic postischemic heart failure. *Circulation* 106(12 Suppl 1):212-217.
- Chatterjee S, Bish LT, Jayasankar V, Stewart AS, Woo YJ, Crow MT, Gardner TJ, Sweeney HL. 2003. Blocking the development of postischemic cardiomyopathy with viral gene transfer of the apoptosis repressor with caspase recruitment domain. *J Thorac Cardiovasc Surg* 125:1461-1469.

- Cohn JN, Bristow MR, Chien KR, Colucci WS, Frazier OH, Leinwand LA, Lorell BH, Moss AJ, Sonnenblick EH, Walsh RA, Mockrin SC, Reinlib L. 1997. Report of the National Heart, Lung, and Blood Institute Special Emphasis Panel on Heart Failure Research. *Circulation* 95:766-770.
- Corrado D, Thiene G. 2006. Arrhythmogenic right ventricular cardiomyopathy/dysplasia: Clinical impact of molecular genetic studies [comment]. *Circulation* 113:1634-1637.
- Dalal D, Nasir K, Bomma C, Prakasa K, Tandri H, Piccini J, Roguin A, Tichnell C, James C, Russell SD, Judge DP, Abraham T, Spevak PJ, Blumenthal DA, Calkin H. 2005. Arrhythmogenic right ventricular dysplasia: A United States experience. *Circulation* 112:3823-3832.
- Dambach DM, Lannon A, Sleeper MM, Buchanan J. 1999. Familial dilated cardiomyopathy of young Portuguese water dogs. *J Vet Intern Med* 13:65-71.
- Diedrichs H, Hagemeister J, Chi M, Boelck B, Muller-Ehmsen J, Schneider CA. 2007. Activation of the calcineurin/NFAT signalling cascade starts early in human hypertrophic myocardium. *J Int Med Res* 35:803-18.
- Dieterle T, Meyer M, Gu Y, Belke DD, Swanson E, Iwatate M, Hollander J, Peterson KL, Ross J Jr, Dillmann WH. 2005. Gene transfer of a phospholamban-targeted antibody improves calcium handling and cardiac function in heart failure. *Cardiovasc Res* 67:678-688.
- Fox PR, Maron BJ, Basso C, Liu SK, Thiene G. 2000. Spontaneously occurring arrhythmogenic right ventricular cardiomyopathy in the domestic cat: A new animal model similar to the human disease. *Circulation* 102:1863-1870.
- Gregorevic P, Blankinship MJ, Allen JM, Crawford RW, Meuse L, Miller DG, Russell DW, Chamberlain JS. 2004. Systemic delivery of genes to striated muscles using adeno-associated viral vectors. *Nat Med* 10:828-834.
- Harvey AM, Battersby IA, Faena M, Fews D, Darke PG, Ferasin L. 2005. Arrhythmogenic right ventricular cardiomyopathy in two cats. *J Small Anim Pract* 46:151-156.
- Hasenfuss G. 1998. Animal models of human cardiovascular disease, heart failure and hypertrophy. *Cardiovasc Res* 39:60-76.
- Ho KK, Pinsky JL, Kannel WB, Levy D. 1993. The epidemiology of heart failure: The Framingham Study. *J Am Coll Cardiol* 22:6A-13A.
- Hoshijima M, Ikeda Y, Iwanaga Y, Minamisawa S, Date MO, Gu Y, Iwatate M, Li M, Wang L, Wilson JM, Wang Y, Ross J Jr, Chien KR. 2002. Chronic suppression of heart-failure progression by a pseudophosphorylated mutant of phospholamban via in vivo cardiac rAAV gene delivery. *Nat Med* 8:864-871.
- Hughes SE, McKenna WJ. 2005. New insights into the pathology of inherited cardiomyopathy. *Heart* 91:257-264.
- Inagaki K, Fuess S, Storm TA, Gibson GA, Mctiernan CF, Kay MA, Nakai H. 2006. Robust systemic transduction with AAV9 vectors in mice: Efficient global cardiac gene transfer superior to that of AAV8. *Mol Ther* 14:45-53.
- Kaye DM, Prevolos A, Marshall T, Byrne M, Hoshijima M, Hajjar R, Mariani JA, Pepe S, Chien KR, Power JM. 2007. Percutaneous cardiac recirculation-mediated gene transfer of an inhibitory phospholamban peptide reverses advanced heart failure in large animals. *J Am Coll Cardiol* 50:253-260.
- Kittleson MD, Meurs KM, Munro MJ, Kittleson JA, Liu SK, Pion PD, Towbin JA. 1999. Familial hypertrophic cardiomyopathy in Maine coon cats: An animal model of human disease. *Circulation* 99:3172-3180.
- Lennon NJ, O'Reilly C, Ohlendieck K. 2000. Impaired Ca²⁺-ATPase oligomerization and increased phospholamban expression in dilated cardiomyopathy. *Int J Mol Med* 6:533-538.
- Lyon AR, Sato M, Hajjar RJ, Samulski RJ, Harding SE. 2008. Gene therapy: Targeting the myocardium. *Heart* 94:89-99.
- MacDonald KA, Kittleson MD, Larson RF, Kass P, Klose T, Wisner ER. 2006. The effect of ramipril on left ventricular mass, myocardial fibrosis, diastolic function, and plasma neurohormones in Maine coon cats with familial hypertrophic cardiomyopathy without heart failure. *J Vet Intern Med* 20:1093-1105.
- MacDonald KA, Kittleson MD, Kass PH, Meurs KM. 2007. Tissue Doppler imaging in Maine coon cats with a mutation of myosin binding protein C with or without hypertrophy. *J Vet Intern Med* 21:232-237.
- Manning BS, Shotwell K, Mao L, Rockman HA, Koch WJ. 2000. Physiological induction of a beta-adrenergic receptor kinase inhibitor transgene preserves ss-adrenergic responsiveness in pressure-overload cardiac hypertrophy. *Circulation* 102:2751-2757.
- Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, Ozelo MC, Hoots K, Blatt P, Konkle B, Dake M, Kaye R, Razaan M, Zajko A, Zehnder J, Rustagi PK, Nakai H, Chew A, Leonard D, Wright JF, Lessard RR, Sommer JM, Tigges M, Sabatino D, Luk A, Jiang H, Mingozzi F, Couto L, Ertl HC, High KA, Kay MA. 2006. Successful transduction of liver in hemophilia by AAV-factor IX and limitations imposed by the host immune response. *Nat Med* 12:342-347.
- Marron BJ. 2002. Hypertrophic cardiomyopathy: A systemic review. *JAMA* 287:1308-1320.
- Meurs KM, Miller MW, Wright NA. 2001a. Clinical features of dilated cardiomyopathy in Great Danes and results of a pedigree analysis: 17 cases (1990-2000). *JAVMA* 218:729-732.
- Meurs KM, Magnon AL, Spier AW, Miller MW, Lehmkuhl LB, Towbin JA. 2001b. Evaluation of the cardiac actin gene in Doberman Pinschers with dilated cardiomyopathy. *Am J Vet Res* 62:33-36.
- Meurs KM, Sanchez X, David RM, Bowles NE, Towbin JA, Reiser PJ, Kittleson JA, Munro MJ, Dryburgh K, MacDonald KA, Kittleson MD. 2005. A cardiac myosin binding protein C mutation in the Maine coon cat with familial hypertrophic cardiomyopathy. *Hum Mol Genet* 14:3587-3593.
- Meurs KM, Lacombe VA, Dryburgh K, Fox PR, Reiser PR, Kittleson MD. 2006. Differential expression of the cardiac ryanodine receptor in normal and arrhythmogenic right ventricular cardiomyopathy canine hearts. *Hum Genet* 120:111-118.
- Meurs KM, Ederer MM, Stern JA. 2007a. Desmosomal gene evaluation in boxers with arrhythmogenic right ventricular cardiomyopathy. *Am J Vet Res* 68:1338-1341.
- Meurs KM, Norgard MM, Ederer MM, Hendrix KP, Kittleson MD. 2007b. A substitution mutation in the myosin binding protein C gene in ragdoll hypertrophic cardiomyopathy. *Genomics* 90:261-264.
- Minamisawa S, Hoshijima M, Chu G, Ward CA, Frank K, Gu Y, Martone ME, Wang Y, Ross J, Kranias EG, Giles WR, Chien KR. 1999. Chronic phospholamban-sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy. *Cell* 96:313-322.
- Most P, Pleger ST, Volkens M, Heidt B, Boerries M, Weichenhan D, Löffler E, Janssen PM, Eckhart AD, Martini J, Williams ML, Katus HA, Remppis A, Koch WJ. 2004. Cardiac adenoviral S100A1 gene delivery rescues failing myocardium. *J Clin Invest* 114:1550-1563.
- NRC [National Research Council]. 1996. Guide for the Care and Use of Laboratory Animals, 7th ed. Washington: National Academy Press.
- Olivetti G, Abbi R, Quaini F, Kajstura J, Cheng W, Nitahara JA, Quaini E, Di Loreto C, Beltrami CA, Krajewski S, Reed JC, Anversa P. 1997. Apoptosis in the failing human heart. *N Engl J Med* 336:1131-1141.
- Oxford EM, Everitt M, Coombs W, Fox PR, Kraus M, Gelzer AR, Saffitz J, Taffet SM, Moise NS, Delmar M. 2007. Molecular composition of the intercalated disc in a spontaneous canine animal model of arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Heart Rhythm* 4:1196-1205.
- Patterson DF. 1978. Lesion-specific genetic factors in canine congenital heart diseases: Patent ductus arteriosus in poodles, defects of the conotruncal septum in the Keeshond. *Birth Defects Orig Artic Ser* 14:315-347.
- Patterson DF, Detweiler DK. 1967. Hereditary transmission of patent ductus arteriosus in the dog. *Am Heart J* 74:289-290.
- Patterson DF, Pyle RL, Van Mierop L, Melbin J, Olson M. 1974. Hereditary defects of the conotruncal septum in Keeshond dogs: Pathologic and genetic studies. *Am J Cardiol* 34:187-205.
- Patterson DF, Haskins ME, Jezyk PF. 1982. Models of human genetic disease in domestic animals. *Adv Hum Genet* 12:263-339.
- Patterson DF, Haskins ME, Schnarr WR. 1981. Hereditary dysplasia of the pulmonary valve in beagle dogs: Pathologic and genetic studies. *Am J Cardiol* 47:631-641.
- Patterson DF, Pexieder T, Schnarr WR, Navratil T, Alaili R. 1993. A single major-gene defect underlying cardiac conotruncal malformations interferes with myocardial growth during embryonic development: Studies in the CTD line of Keeshond dogs. *Am J Hum Genet* 52:388-397.

- Pleger ST, Most P, Boucher M, Soltys S, Chuprun JK, Pleger W, Gao E, Dasgupta A, Rengo G, Remppis A, Katus HA, Eckhart AD, Rabinowitz JE, Koch WJ. 2007. Stable myocardial-specific AAV6-S100A1 gene therapy results in chronic functional heart failure rescue. *Circulation* 115:2506-2515.
- Ponder KP, Haskins ME. 2007. Gene therapy for mucopolysaccharidosis. *Expert Opin Biol Ther* 7:1333-1345.
- Pyle RL, Patterson DF, Chacko S. 1976. The genetics and pathology of discrete subaortic stenosis in the Newfoundland dog. *Am Heart J* 92:324-334.
- Richardson P, McKenna W, Bristow M, Maisch B, Mautner B, O'Connell J, Olsen E, Thiene G, Goodwin J, Gyarfás I, Martin I, Nordet P. 1996. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies. *Circulation* 93:841-842.
- Sasano T, Kikuchi K, McDonald AD, Lai S, Donahue JK. 2007. Targeted high-efficiency, homogeneous myocardial gene transfer. *J Mol Cell Cardiol* 42:954-961.
- Sherrid MV. 2006. Pathophysiology and treatment of hypertrophic cardiomyopathy. *Prog Cardiovasc Dis* 49:123-151.
- Sleeper MM, Henthorn PS, Vijayasathy C, Dambach DM, Bowers T, Tijsskens P, Armstrong CF, Lankford EB. 2002. Dilated cardiomyopathy in juvenile Portuguese water dogs. *J Vet Intern Med* 16:52-62.
- Thiene G, Basso C, Calabrese F, Angelini A, Valente M. 2005. Twenty years of progress and beckoning frontiers in cardiovascular pathology: Cardiomyopathies. *Cardiovasc Pathol* 14:165-169.
- Van Mierop LH, Patterson DF. 1978. The pathogenesis of spontaneously occurring anomalies of the ventricular outflow tract in Keeshond dogs: Embryologic studies. *Birth Defects Orig Artic Ser* 14:361-375.
- Van Mierop LH, Patterson DF, Schnarr WR. 1977. Hereditary conotruncal septal defects in Keeshond dogs: Embryologic studies. *Am J Cardiol* 40:936-950.
- Vinge LE, Raake PW, Koch WJ. 2008. Gene therapy in heart failure. *Circ Res* 102:1458-1470.
- Wang Z, Kuhr CS, Allen JM, Blankinship M, Gregorevic P, Chamberlain JS, Tapscott SJ, Storb R. 2007. Sustained AAV-mediated dystrophin expression in a canine model of Duchenne muscular dystrophy with a brief course of immunosuppression. *Mol Ther* 15:1160-1166.
- Wang Z, Tapscott SJ, Chamberlain JS, Storb R. 2009. Gene therapy in large animal models of muscular dystrophy. *ILAR J* 50:187-198.
- Werner P, Raducha MG, Prociuk U, Budarf M, Henthorn PS, Patterson DF. 1999. Comparative mapping of the DiGeorge region in the dog and exclusion of linkage to inherited canine conotruncal heart defects. *J Hered* 90:494-498.
- Werner P, Raducha MG, Prociuk U, Ostrander EA, Spielman RS, Kirkness EF, Patterson DF, Henthorn PS. 2005. The Keeshond defect in cardiac conotruncal development is oligogenic. *Hum Genet* 116:368-377.
- Werner P, Raducha MG, Prociuk U, Sleeper MM, Van Winkle TJ, Henthorn PS. 2008. A novel locus for dilated cardiomyopathy maps to canine chromosome 8. *Genomics* 91:517-521.
- Woo YJ, Zhang JC, Taylor MD, Cohen JE, Hsu VM, Sweeney HL. 2005. One year transgene expression with adeno-associated virus cardiac gene transfer. *Int J Cardiol* 100:421-426.
- Wright MJ, Wightman LM, Lilley C, de Alwis M, Hart SL, Miller A, Coffin RS, Thrasher A, Latchman DS, Marber MS. 2001. In vivo myocardial gene transfer: Optimization, evaluation and direct comparison of gene transfer vectors. *Basic Res Cardiol* 96:227-236.