Principles of Genetic Murine Models for Cardiac Disease

Katherine E. Yutzey, PhD; Jeffrey Robbins, PhD

Abstract—It was only ≈15 years ago that methodologies evolved to the point where we began to manipulate the genetic apparatus of the mouse such that proteins of the investigator’s choice could be expressed in a 4-chambered, mammalian heart. Our abilities to express both normal and mutated proteins in the heart or to create genetic nulls in which the protein is not expressed at all continue to evolve. With the tools now available, one can target protein expression to the different cell types present in the heart, often at a particular time, and, in some cases, turn off the protein as development progresses or the animal ages. These abilities have enabled us to model many of the genetic mutations identified as causative for pediatric and/or adult cardiovascular disease and heart failure. Identifying the primary genetic cause is, more often than not, insufficient for designing effective therapeutics or interventions. Therefore, it is critical to be able to develop animal models that accurately recapitulate the pathogenic processes that ensue as a result of mutant gene expression or loss of protein expression. In this review, we discuss the nature, strengths, and weaknesses of the current set of tools for developing genetically manipulated mouse models, as well as the relevance of these models for understanding cardiovascular disease and illuminating potential therapeutic avenues. (Circulation. 2007;115:792-799.)

Key Words: cardiovascular diseases • genes • molecular biology

The genetic abnormalities that underlie or predispose one toward congenital heart disease, the hypertrophic and dilated cardiomyopathies, vascular disease, cardiac arrhythmias, myocarditis, and other pathologies that are the basis of cardiovascular disease (CVD) are being rapidly defined. The genomes of >350 organisms have now been sequenced, and the promise of personal sequences of one’s own genome at reasonable cost is on the near horizon. Unfortunately, the knowledge that one has a mutation that predisposes or causes CVD cannot translate directly into a treatment, although it can help in risk stratification. The design of effective therapeutics often requires an understanding of the pathological process or processes that occur as a result of the expression of a dominant or recessive gene product. Furthermore, to fully understand the physiological consequences requires understanding the physiological networks that are perturbed as a result of the expression of the mutation or lack of the gene’s product. For these reasons, there has been a drive to model the defined mutations in animal models so that the effects can be studied during cardiac development, maturation, and aging at the molecular, biochemical, cellular, organ, and whole animal levels.

Manipulating the Mouse Genome

The mouse has been the model of choice for creating genetic models of human CVD. Using animal models can establish causality, confirm proof-of-principal for a particular therapeutic approach, and help people to understand the multifactorial bases for CVD, including the interaction between environmentally acquired factors, such as viral infection, and a genetic predisposition to disease.1 Because the basic gene regulatory networks are often conserved between disparate organisms, insights into the underlying mechanisms driving heart development and function can be gained from studying such organisms as flies, worms, and fish.2 However, the advantages of dealing with a mammalian, 4-chambered heart in terms of the application of the data to human CVD remain compelling. With the development of our abilities to manipulate the cardiovascular protein complement by genetic manipulation of the mouse genome, the mouse became and remains firmly established as the favored model in which to make defined genetic changes to study the causes and mechanistic bases for human CVD. Two complementary techniques are used for producing murine models of CVD. Although often referred to interchangeably, they are distinct methodologies that result in very different outcomes in terms of the genetic complement (Table). Transgenesis involves the injection of naked DNA containing the gene sequence of choice into the nucleus of a fertilized 1-cell embryo. Thus, the endogenous gene is not affected, and no phenotype will be detectable unless the product of the transgene is dominant. In contrast, gene targeting depends on an exceedingly rare event in the mammalian genome, homologous recombination, in which a suitably modified DNA is inserted into the endogenous genetic site and replaces the normal sequence. An additional difference is that conventional gene targeting depends on homologous recombination of the electroporated DNA into totipotent embryonic stem cells.3 These targeted cells are then microinjected into an early embryo, usually one

From the Division of Molecular Cardiovascular Biology, Department of Pediatrics, Children’s Hospital Research Foundation, Cincinnati, Ohio.
Correspondence to Jeffrey Robbins, PhD, Division of Molecular Cardiovascular Biology, 3333 Burnet Ave, Cincinnati, OH 45229-3039. E-mail jcf.robbins@chcmc.org
© 2007 American Heart Association, Inc.
Circulation is available at http://www.circulationaha.org
DOI: 10.1161/CIRCULATIONAHA.106.682534
at the 16-cell blastocyst stage. The technique is most often used to create a null allele, that is, to “knock out” a gene, although in a more technically demanding series of targetings, single base pair mutations can actually be “knocked in” (to) the gene of choice. By directing expression of an engineered protein to the heart, one is now able to effectively remodel the cardiac protein profile and study the consequences of a single genetic manipulation at the molecular, biochemical, cytological, and physiological levels, under both normal and stress stimuli.

Technical advances have focused on making both transgenesis and gene targeting more precise. For example, systemic expression of a transgene can seriously complicate any resultant cardiac phenotype. The first cardiac-specific example of transgenic gene expression was a serendipitous event in which, despite having strong, non–cell-specific transcriptional activation elements, the construct was expressed only in the heart. However, publication of these data illustrated the potential power of cardiac-specific expression of a transgene and prompted one of us (J.R.) to develop reagents capable of driving cardiomyocyte-specific transgene expression. The development of promoters capable of driving cardiomyocyte-specific expression at different developmental times in the heart has enhanced the utility of the transgenic approach for studying CVD in the absence of confounding effects on other organ or muscle systems. Not surprisingly, the transcriptional regulatory sequences of the cardiac contractile genes themselves have been used effectively to drive cardiac transgenic expression. These include the actin, myosin light chain 2v, and α- and β-myosin heavy chain promoters, among others. Literally hundreds of transgenes affecting the electric, mechanical, transport, and metabolic properties, as well as channels, calcium cycling, and structural aspects of cardiac function have been expressed with the use of these cardiac-specific promoters, and models for general events such as hypertrophy or specific cardiac diseases have been made. Although cardiac-specific transgenesis forms the basis for a majority of the murine models of CVD, it remains a relatively blunt instrument because many of the models express the transgene at very high levels, raising the possibility of nonphysiological consequences due to the aberrant levels of gene expression. Therefore, it is always prudent to interpret any resultant phenotype cautiously and preferably to compare those animals to animals in which a transgene encoding the normal protein is expressed at similar levels so as to have a true control for the experiment. In the case in which ectopic proteins are expressed, the relevant controls are even more difficult, and the data must be interpreted in a conservative manner.

Although animal models of human CVD have been invaluable, the clinician is most interested in whether the resultant cardiac pathology is controllable or reversible. To that end, efforts have been devoted to allowing more precise control of transgene induction, preferably by pharmacological means, and tetracycline has been used to reversibly control transgene expression. The drug interacts with a transcriptional activating protein in such a manner as to either activate or, in some cases, inactivate its ability to bind to a promoter region and initiate transcription of a transgene. Other inducible systems have also been adopted successfully for transgenic use, but these systems use less innocuous inducers that can have undesired pleiotropic effects on the animals. At this point, the tetracycline-based systems have clear advantages for animal-based studies in that the required drug treatment is minimally intrusive on the animals’ general physiology. These systems have now been developed and validated for inducible and reversible cardiomyocyte-specific transgene expression and detailed protocols that render the technology accessible have been published. Thus, it is now possible to target transgenic expression very precisely in the cardiomyocyte, controlling onset, duration, and dose by pharmacological means.

Gene targeting is genetically more precise than transgenesis because, in the latter, transgene insertion and copy number cannot be controlled, at least if conventional transgenic techniques are used. Many genes whose protein products are important for normal cardiac development and function have been ablated, but this technique can also be a blunt instrument for understanding the precise mechanisms involved in the ensuing pathology. Frequently, the targeted genes encode polypeptides that are involved pleiotropically in cardiac development or basic anatomy. Although the ablation can have a major impact on the heart, the deficit/remodeling phenotype may not be a primary consequence of the null mutation but rather is a secondary consequence of a more

---

### Comparison of Gene Targeting and Transgenesis in the Mouse

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Transgenesis</th>
<th>Gene Targeting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>7–15 wk</td>
<td>0.8–1.5 y</td>
</tr>
<tr>
<td>Cost, $</td>
<td>1500–6000</td>
<td>15 000–30 000</td>
</tr>
<tr>
<td>DNA used</td>
<td>Additional DNA is inserted into the genome</td>
<td>Genes are manipulated within the endogenous chromosomal context</td>
</tr>
<tr>
<td>Reversibility</td>
<td>Transgene expression can be controlled reversibly, turning it on and off by pharmacological intervention</td>
<td>The targeting event can be controlled in a cell type–specific and temporal manner, but the event is irreversible</td>
</tr>
<tr>
<td>Precision</td>
<td>Neither insertion site nor copy number can be controlled; this can lead to insertional mutagenic events or atypically high levels of expression, and multiple lines must be analyzed</td>
<td>The genetic event is precise and occurs at a single site; if a particular mutation is being made, the mutated gene is expressed under the control of the endogenous promoter</td>
</tr>
<tr>
<td>Gene activity</td>
<td>With certain cardiac-specific promoters, expression levels are copy number dependent</td>
<td>Null or site-specific mutant alleles can be made and recessive mutations studied</td>
</tr>
</tbody>
</table>

Shown are some of the considerations that dictate an investigator’s approach. The most precise genetic manipulation, cell-specific placement of a mutation within the endogenous genetic context (inducible, conditional knockin technology), is used infrequently because of its technical difficulty, long time frame, and high cost. The wide variation in cost is due to different support structures at various institutional cores or the use of a commercial source.
Cardiac-specific targeting strategies use cardiomyocyte-specific expression of a recombinase, Cre, that can recognize short sequences consisting of two 13-bp inverted repeats separated by an 8-bp asymmetrical spacer region, termed a loxP site. With the use of homologous recombination, these sites are placed into the locus such that they flank the gene target of choice. Cre activity then excises the gene fragment, creating the targeted allele. Although technically more complex, the increased precision of the procedure, which is termed conditional gene deletion, is usually worth the extra effort, and, if one makes cre expression inducible by flanking the cre sequence with mutant estrogen domains (Mer) that are insensitive to endogenous levels of 17β-estradiol but sensitive to the estrogen antagonist tamoxifen,25 the gene-targeting event can also be controlled temporally in the cardiomyocyte population. Again, one must use caution in designing and interpreting these results because high levels of cre expression can, in some cases, lead to CVD.24

**Mouse Models and Their Translation to Human Disease**

A listing of all of the murine models used in gain- and loss-of-function experiments focused on the cardiovascular system is clearly beyond the scope of this short review or even a more comprehensive one. Indeed, such a review would be essentially a multipage catalog of the literally thousands of articles that deal with expression of normal or mutated proteins in the cells normally found in the heart or the loss of their expression and the physiological consequences at the molecular, cellular, and whole-organ levels. Murine models have been used to prove or disprove causality, necessity, and sufficiency of various proteins or their absence in causing cardiac pathology and failure. Cardiac function, remodeling, cell death, and proliferation have all been explored at the single-protein level. These studies (currently ~4000 are listed in the “Animal Models of Human Disease” collection available at the *Circulation Research* Web site: http://circres.ahajournals.org/cgi/collection) have led to many basic insights into cardiac structure and function.

Thus, the study of genetically engineered mice generated through transgenosis or gene targeting has led to the identification of specific molecular mechanisms or genetic lesions that cause adult and congenital CVD. Significant advances in the understanding of the molecular relationships and feedback mechanisms that underlie cardiac function and dysfunction have been made in the past decade through analysis of mice with gain or loss of function of specific signaling molecules, transcription factors, or structural proteins.25 Genetic manipulations that recapitulate human CVD-causing mutations have been introduced in mice, and the resulting heart malformations or pathogenic processes have been examined in a controlled experimental setting.26 Importantly, these models have led to an iterative process, in which a gene mutation may first be noted in either a patient or a mouse. The genetic lesions are initially modeled in the mouse but subsequently lead to screening of families with CVD with the use of high-throughput sequencing of candidate genes selected as a result of the murine studies (Figure 1). This general approach has begun to yield novel insights into the genetic basis of heart development and disease.27 The extensive work in genetically engineered mice has prompted ongoing studies aimed at defining potential therapeutic targets amenable to conventional small molecule–based pharmaceuticals or more novel approaches such as stem cell–based therapies.

However, despite these impressive advances using the mouse models and the many conferences trumpeting their imminent translation into clinical practice, these studies generally have not yet resulted in significant changes in clinical practice. Although disappointing, it is understandable when one considers the time it takes to develop an effective drug even when the causative gene has been identified and the mechanistic implications of the mutation have been defined. Understanding the basic cause of a disease and even the mechanisms of the ensuing pathology does not translate immediately into an effective treatment or a cure. For example, we have known the genetic cause(s) of sickle cell disease for almost 50 years, and there is still no effective cure.
Murine-based data have confirmed causality, led to improved genetic testing,28 and prompted counseling for avoidance of certain behaviors that can trigger a fatal cardiac event, but they have not yet led to the development of an effective treatment. Below, we will focus on a few examples that illustrate how murine models have been used to develop concepts that have placed us on the brink of changing the clinical outcome of human CVD.

**β-Adrenergic Receptors and Heart Failure**

β-Adrenergic receptors (β-ARs) regulate both heart rate and contractility through their interactions with catecholamines such as norepinephrine and epinephrine.29,30 There are 3 subtypes, β1, β2, and β3, and each has unique functions.31 β2-AR represents the most abundant subtype in the heart, comprising ≈80% of the total AR receptors.32 The failing human heart is chronically stimulated, and, although this may be salutary in maintaining normal hemodynamics, chronic adrenergic stimulation is a harmful compensatory response, exacerbating the downward spiral toward failure and death. Recognition of this has led to a fundamental shift in the treatment paradigm, and it is now well documented that long-term β-AR blockade is an effective clinical treatment.33–35 The use of transgenic and gene-targeted models has enabled investigators to define the roles the different AR subtypes play in developing cardiac disease, pointed the way to novel and more effective therapeutic interventions, and uncovered the mechanisms that underpin the paradigm shift from short-term conservation of cardiac hemodynamics to the long-term maintenance and reparative therapeutic strategy of β-AR blockade.35

A reduction in β-AR density and desensitization in heart failure patients was demonstrated almost 25 years ago.36 Therefore, manipulation of neurohumoral stimulation of cardiac contractility was an attractive, early target when the necessary reagents for genetic alteration of the protein complement of the heart became available. The first study detailing a murine model in which a β-AR was manipulated used the cardiomyocyte-specific α-myosin heavy chain promoter37 to express the β1-AR at very high levels, ≈200-fold that of endogenous expression. High levels of transgenic expression of even normally innocuous proteins can often cause cardiomyopathy.10,24 but the transgenic hearts showed the expected physiological response, with dramatically elevated cardiac contractility that was unresponsive to additional β-adrenergic stimulation.38 Those effects appeared to be dose dependent because more modest expression levels did not result in increased cardiac contractility. Elevated cardiac function persisted for at least 1 year and was accompanied by only mild fibrosis.38

Clinically derived data indicated that the β-AR subtypes might be functionally divergent. In cardiac disease, β1-AR levels significantly decrease, whereas β2-AR levels are relatively conserved. When it was considered that β2-AR is the dominant subtype on cardiomyocytes, a similar strategy of cardiomyocyte-specific transgenic overexpression of β2-AR was undertaken. In contrast to the very high levels of overexpression achieved with β2-AR, only modest expression levels of 5- to 15-fold were observed in the β1-AR transgensics.70 The mice exhibited enhanced contractility early in life but, in contrast to the β2-AR animals, the β1-AR hearts developed marked hypertrophy, fibrosis, and loss of contractile function as early as 16 weeks, with ejection fractions decreasing to ≈20% by 35 weeks. Other investigators confirmed these observations: High levels of β2-AR overexpression are relatively benign or even beneficial as enhanced inotropy is maintained over a period of months with little or no cardiac pathology resulting, whereas even modest expression of the β1 subtype results in enhanced contractility early on but quickly translates into hypertrophy and drives the heart toward failure.40 Thus, the murine models unequivocally pointed to the functional distinctions between the 2 receptors and provided evidence for the potential efficacy of more precise targeting for β1-blockade in the treatment of human heart failure.

Murine models have been instrumental in identifying potentially “druggable” processes or even specific targets, and G-protein–coupled receptors (GPCRs), such as β-ARs, are attractive candidates. As might be expected for such potent signalers, β-ARs are subject to precise regulation. One of the most important mechanisms for desensitization is phosphorylation of the agonist-occupied receptors by a family of kinases termed GPCR kinases or GRKs.41 The β-AR kinases (eg, βARK1 [GRK2]), will only phosphorylate agonist-occupied β-ARs, and a number of these kinases are expressed in the heart. The cardiac β-ARs are desensitized in part through the action of these enzymes, which can also act on other GPCRs that are present in the heart.42 βARK manipulation in murine models thus became a compelling avenue to explore, and cardiomyocyte-targeted overexpression of βARK1 and a βARK1 inhibitor, termed βARKct, quickly followed the initial β-AR overexpression studies.43 βARK levels are often elevated in heart failure, and overexpression of βARK resulted in a blunted inotropic response to isoproterenol infusion and reduced functional coupling of β-ARs. βARK inhibitor overexpression resulted in increased contractility at baseline and in response to isoproterenol, confirming the physiological importance of the protein. Strikingly, when the βARKct mice were crossed to genetically altered mice that developed heart failure and exhibited elevated βARK levels and receptor uncoupling, βARK inhibition was able to either rescue the phenotype completely or significantly prolong survival and improve cardiac function. This effect was augmented by the simultaneous treatment of the animals with the β-antagonist metoprolol.45 These and other extensive data gathered from mouse models that either overexpress or genetically lack components of the AR axis have identified a number of potential targets, as well as genetic polymorphisms that may be useful for personalized pharmacogenetic approaches.46 Multiple pharmaceutical companies are pursuing these for potential therapeutic applications, with phase I clinical trials anticipated in the near future.

**Clinical Applications of Mouse Models of Congenital Heart Disease**

Congenital heart defects occur in ≈1 of 100 live births in the United States and are a leading cause of mortality in the first year of life.47 Treatment of severe congenital heart defects is often limited to surgical intervention, and improvement in this area has led to an increased incidence of congenital heart defects in older individuals.48 Although recent studies in mice and other animal model systems have increased our understanding of both the candidate disease genes and underlying
mechanisms, the embryonic origin of many congenital malformations, which often first manifest themselves during the first trimester, has impeded development of therapeutic interventions. However, several gene loci associated with specific cardiac malformations have been identified, and this has led to more effective genetic counseling and management of congenital heart defects in affected individuals. Below we illustrate examples of the manner in which murine models are being used to develop potential treatments for congenital heart defects.

**Down Syndrome**

Down syndrome (DS), which is caused by trisomy of chromosome 21 (Ts21), is the most common genetic cause of congenital heart defects. Congenital heart defects occur in >40% of DS infants and are the leading cause of mortality in the first year of life. The cardiovascular malformations associated with DS include atrioventricular septal defects, ventricular septal defects, atrial septal defects, and tetralogy of Fallot. In general, trisomy of genes on chromosome 21 leads to increased expression of the encoded proteins, which has traditionally been thought to be the mechanism underlying DS phenotypes. Extensive research with a variety of animal models provides evidence that DS results from large-scale chromosomal anomalies and involves many genes rather than being caused by elevated expression of a select number of triplicated alleles. Although it may be difficult to pinpoint the precise molecular lesions that cause specific developmental anomalies, murine models are now being used to identify therapeutic interventions for DS-related disease processes.

Genetically altered mice with trisomy of regions of mouse chromosomes syntenic to human chromosome 21 (Ts16 and Ts65Dn) or with Ts21 sequences have been used to examine genetic mechanisms of DS (Figure 2). Somewhat disappointingly, the mouse models generated with trisomy of mouse chromosomal segments syntenic to human chromosome 21 do not fully recapitulate the DS phenotype and therefore are not viewed as optimal disease models. Ts16 mice die during gestation from severe cardiovascular malformations, whereas Ts65Dn mice exhibit rare (<5%) and relatively mild congenital heart defects, with neither reflecting the human DS phenotype. Recently, Tc1 mice engineered with a nearly complete copy of human chromosome 21 have been reported and are the first animal model to exhibit high-frequency (>50%) atrioventricular canal defects characteristic of DS, in addition to other features. These mice represent the best animal model for human Ts21 to date and will likely provide new opportunities for identification of molecular mechanisms and therapies for DS patients.

The Ts65Dn mouse has already been used to test potential therapeutics for brain developmental defects neonatally and in adults. Treatment of newborn Ts65Dn mice with a small molecule agonist of the Hedgehog signaling pathway restored cerebellar precursor cell populations. In adult Ts65Dn mice, treatment with the serotonin selective reuptake inhibitor fluoxetine rescued deficient neurogenesis in the hippocampus, which is associated with behavioral deficits. These studies have not yet been translated to the clinic, but, in contrast to previously advocated therapies based on nutritional supplements or piracetam, the mouse models finally provide a rational basis for clinical trials of novel evidence-based therapeutics. The Tc1 mice with DS-related cardiac anomalies should provide similar opportunities for pharmaceutical manipulation of clinically important CVD mechanisms.

**Marfan Syndrome**

Marfan syndrome is an autosomal dominant genetic disorder caused by mutation of the extracellular matrix (ECM) gene fibrillin-1 (fbln1). Individuals with Marfan syndrome exhibit connective tissue disorders of the cardiovascular system, such as aortic dissection and mitral valve prolapse, in addition to skeletal abnormalities, ocular lens dislocation, and lung pathology. Fibrillin-1 belongs to a family of microfibril proteins that are major structural components of ECM but inhibit connective tissue disorders of the cardiovascular system. Further CVD mechanisms, the most common cause of premature death with Marfan syndrome, when untreated, is acute aortic dissection, but mitral valve disease is the most serious indication for surgery or mortality in young children. Current treatment of severe aortic and cardiac disease associated with Marfan syndrome is primarily surgical, but animal model systems have provided the mechanistic foundation for new therapeutic strategies.

A mutation corresponding to a prevalent Marfan syndrome allele encoding a cysteine substitution for glycine, C1039G, was engineered into the mouse fbln1 gene to generate an animal model of the human condition. Mice heterozygous for this fbln1(C1039G) allele exhibit ECM abnormalities and degeneration of the aortic wall characteristic of Marfan syndrome. Further
analysis of the molecular pathogenesis of these animals revealed that TGF-β signaling was elevated with compromised fibrillin-1 function. Mitral valves of \(fbn1^{+/+}\) mice become thickened in the postnatal period, and functional studies showed prolapse and regurgitation in the adults. Molecular analyses of the affected valves demonstrated that TGF-β activity and signaling were elevated and that TGF-β neutralizing antibodies inhibited valve pathogenesis. These studies demonstrated that pathogenesis of heart valves associated with Marfan syndrome is dependent on elevation of TGF-β signaling pathways. Losartan, a Food and Drug Administration–approved angiotensin type 1 antagonist, inhibits TGF-β signaling and is used clinically as a treatment for hypertension. The efficacy of this drug in ameliorating aortic wall degeneration and dilation was examined in \(fbn1^{+/+}\) mice. Strikingly, losartan treatment restored aortic wall architecture and prevented aortic dilatation in \(fbn1^{+/+}\) mutant mice, which was not observed with \(β\)-adrenergic blocking agents. It is notable that this model is based on inhibition of signaling mechanisms of pathogenesis rather than a gene therapy approach, and it is likely that this paradigm may be extendable to many different cardiac malformations and functional deficits that increase in severity over time. The validation of losartan in the Marfan syndrome mouse therefore represents an exciting potential therapeutic approach for clinical management of Marfan syndrome and related conditions. Future animal studies and human trials will be necessary to translate these molecular studies into clinical practice.

**Murine Models: Caveats and Limitations**

Although the current models have undeniably taught us a tremendous amount about basic cardiovascular cell biology and physiology, they are merely the first iteration of a long process of modeling cardiovascular function and disease. It is incumbent on the individual investigator to treat the data conservatively, particularly when they are being applied to development of a potential therapeutic or clinical trial design. As noted above, transgenesis can often lead to developmentally inappropriate expression or to very high expression levels of a protein that is normally present in very low amounts, resulting in side reactions and artificial physiological responses that are fundamentally misleading. Similarly, gene ablations can often result in the production of unexpected protein fragments or hypomorphic alleles that, if not accounted for, will result in the misinterpretation of the resultant changes in cardiovascular physiology. The devil lies in the details, and the details have been and are often overlooked in the first rush to study all of the fascinating phenotypes. However, these may not fully represent or may even misrepresent relevant applications to human disease as the investigators focus on a particular pathway and set of downstream signaling events. Technical details of how the genetic manipulation was exactly performed are often overlooked or superficially described, and yet an exact description of the process is often critical for understanding the resultant phenotype. For example, many of the existing knockout models have been made such that expression of neighboring loci may also be affected, complicating interpretation of the data. Indeed, when “null” mutants of the myogenic regulatory factor MRF4 were constructed by 3 different laboratories, 3 phenotypes resulted, ranging from complete viability to embryonic lethality. The different phenotypes appear to be a result of the exact location in which gene disruption was targeted and the subsequent effects on other genes in the neighborhood. Although these phenomena can now be largely circumvented by the more precise genetic targeting methodologies used to engineer specific mutations into the genetic locus being targeted, those “newer” mice currently make up only a small fraction of the gene-targeted mice currently available.

It is also critical to remember the basic differences in cardiovascular physiology between mice and humans. Although it is clear that the basic organizational, developmental, and signaling pathways are conserved, and much can be learned from the mouse models, the subtleties of the different proteomes and the way in which the different components interact and differ between mice and humans are critical, particularly when therapeutic approaches are considered. For example, 2 distinct cardiac isoforms of the myosin heavy chain exist and are expressed in a species-dependent manner. The mouse ventricle contains predominantly \(α\)-myosin heavy chain, and the human ventricle contains mostly \(β\)-myosin heavy chain. The different cardiac myosins have been studied for >30 years, and their kinetic, mechanical, and contractile properties are well known: \(α\)-myosin has a higher ATPase activity and a faster maximum velocity of shortening but a lower tension-time integral than the \(β\)-isofrom.69,70 Despite these differences, significant data have accumulated in which the contractile apparatus, including myosin, has been modified, resulting in hypertrophy or dilation followed by failure. For example, a mutation that occurs in human \(β\)-myosin at residue 403 (R403Q) causes familial hypertrophic cardiomyopathy,71 but because of the isoform differences, the mutation was made in the \(α\)-isofrom. Although the model recapitulated aspects of the human disease, it must be appreciated that it was only an approximation,72 and major aspects of the human disease were not reflected in the mouse model. Indeed, we have found that when the 403 mutation is placed into the mouse \(β\)-myosin backbone, it leads to much more severe functional deficits than are apparent in the \(α\)-myosin heavy chain \(R403Q\) mice (M. Krenz, MD, and J. Robbins, PhD, unpublished data, 2004). These kinds of important physiological differences can be found in many of the basic parameters underlying cardiac output,73 cardiac electrophysiology,74 and calcium flux.75 Although these concerns do not negate data gathered with the use of the murine models, they underscore the fact that the murine data should invariably be treated with caution as they are applied to human disease.

**Future Developments: On the Brink**

Despite the limitations outlined above, murine models will continue to play a major role in cardiovascular research as the research and clinical communities translate the basic information into effective therapies. Modeling the genetic defects that lead to CVD will continue to play an important role, but we will continue to struggle with the relatively laborious procedures involved in creating the animals as we consider the flood of genetic information that will ensue as personalized genotyping becomes affordable and the issues of compound heterozygosity are addressed. The precision of both gain- and loss-of-function approaches will continue to improve, and we will be able to direct synthesis of engineered
proteins in even more precise ways to the cells of our choice or even to specific subcellular compartments. Similarly, our ability to knock out or knock down expression of specific genes will become less labor intensive as the global initiatives directed at creating complete banks of ablated murine genes progress or are completed.

Although the emphasis of the last 20 years has been on manipulating the genome, the transcriptome and proteome are both attractive targets, and the technologies to directly affect these populations will continue to develop. This is critical to improving both the precision and effectiveness of our approaches because it is these populations that actually reflect and direct the dynamic states of the cell; both are substantially more complex than the genome in terms of potential informational content. Small, inhibitory RNAs or gene silencing by introduction of short hairpin RNAs offers the potential of tremendous specificity in therapeutic applications, although formidable technical problems remain in terms of both efficacy and delivery. However, the technology is clearly capable of allele-specific knockdown or silencing and should, as the technical issues of stability and delivery are addressed, become the method of choice for creating hearts in which a genetic locus is functionally inactive through post-transcriptional silencing.

As we hope this review has made clear, major strides in understanding CVD have been made through study of the murine models, and we believe that we are on the brink of seeing them translated into more effective therapeutic interventions on both known and newly identified targets. Experimental lines of investigation, which are often initiated by a set of clinical and human genetic data, are now used routinely to create mouse models that prove causality and/or proof of principle for a potential therapeutic intervention. Pathogenic mechanisms can be postulated and confirmed or disproved in murine models, and we believe that we are on the brink of improving both the precision and effectiveness of our approaches.

**Sources of Funding**

This work was supported by National Institutes of Health research grants R01 HL69799, HL60546, HL52318, HL60546, and HL56370 (to Dr Robbins) and R01 HL082716, HL60651, and P50 HL74728 (to Dr Yutzey).

**Disclosures**

None.

**References**


49. Braun T, Arnold HH. Inactivation of Myf-6 and Mef-5 genes in mice leads to alterations in skeletal muscle development. EMBO J. 1995;14:1176–1186.
Principles of Genetic Murine Models for Cardiac Disease
Katherine E. Yutzey and Jeffrey Robbins

Circulation. 2007;115:792-799
doi: 10.1161/CIRCULATIONAHA.106.682534
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/115/6/792

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/