Transgenic and Gene-Knockout Rodents as Research tools for Cardiovascular Disorders

by Kapil Kapoor* & Madhu Dikshit
Cardiovascular Pharmacology Unit, Pharmacology Division, Central Drug Research Institute, Lucknow, India

Summary
Cardiovascular disorders like hypertension, cerebral stroke, heart failure and thromboembolism account for a high degree of morbidity and mortality all over the World. As the regulation of the cardiovascular system is complex, the study of cardiovascular disorders has been limited to whole-organism models. The last decade has witnessed an upsurge in transgenic and gene knockout technologies, which have played a major role in the discovery of a particular gene, or its product, implicated in various cardiovascular disorders. Knockout and transgenic animals are likely to become important tools in drug development to determine the physiological sites of action for newly developed pharmacological agents.

The present review will briefly discuss the methods and types of genetically engineered rodents (transgenic and gene knockout models) with alterations in second messenger systems involved in cardiovascular disorders.

Introduction
Transgenic technology has developed at a fast pace over the past few years. The establishment of embryonic stem cells and the finding that they can serve as a bridge between genetic manipulations in vitro and biological analysis in vivo enabled the systematic creation of animal strains with defined genetic alterations. Many diseases like Parkinson’s disease, Alzheimer’s dementia, hypertension, diabetes and cancer result as a pathological consequence of environmental insult to the cellular genetic control mechanisms. These models are providing novel insights into how the genome and environment interact in vivo. Further, in diseases like dyslipidaemia and atherosclerosis development of a reliable animal model is not easy due to dissimilarity from humans in the regulatory pathways of lipid metabolism (Tailleux et al., 2003). An ideal transgenic animal model expressing human nuclear receptor ligands of interest along with dyslipidaemia, insulin resistance and atherosclerosis could be an exciting research tool.

This review lists the alterations in the genetic makeup of mice and rats to address some of the common human cardiovascular diseases.

Techniques
1. Transgenic technology
Transgenic animals are being used for simulating diseases and testing new therapies. The overexpressed transgene can be a normal gene product, a mutant gene product (increasing or decreasing the activity of the protein), or an antisense RNA, which hybridizes with native RNA and decreases the expression of the normal gene product. Transgenic technology can also be used to eliminate a specific tissue by overexpression of a gene encoding toxins that will destroy the target tissue in which they are expressed. By using specific promoters one can drive tissue-specific or developmental-specific expression of the transgene. For example, a trans-
gene can be expressed in all tissues of the transgenic animal using a promoter from a ubiquitously expressed gene, such as that of β-actin or of the simian virus 40T antigen. Alternatively, selective expression in fat cells or in skeletal muscle can be achieved using the aP2 or MCK promoters respectively, while the insulin promoter restricts the expression of the transgene to pancreatic β-cells (Mauvais-Jarvis and Kahn, 2000). Inducible promoters allow transgene expression at a time chosen by the investigator. The most commonly used method of gene transfer to create transgenic mice is the direct microinjection of the DNA construct into the pronuclei of fertilized eggs (Gordon et al., 1980). The injected eggs are then implanted in the reproductive system of a pregnant mouse. If successful, the transgene integrates randomly into the genome in the one-cell stage and can be transferred to the next generation via germ cells. The level of expression of the transgene and the resulting phenotype of the transgenic mouse are dependent upon a variety of factors, including the number of copies integrated, the position of integration into the genome and the strength of the promoter used (Gordon et al., 1980).

2. Gene knockout technology
The function of a gene product in intact animals can also be investigated by eliminating its expression via homologous recombination targeted gene knockout. A targeting vector is created by flanking a mutated DNA sequence (the gene of interest) with the DNA sequence homologous to the endogenous gene. This vector is then introduced into mouse embryonic stem (ES) cells where the mutant DNA replaces the native gene via homologous recombination. ES cells that have correctly incorporated the mutant DNA are then injected into the blastocyst of a pregnant mouse where they participate in the formation of the tissues of a chimeric animal (Bradley et al., 1984). Those chimeras that carry the mutation in their germ cells can be bred to obtain mice heterozygous and homozygous for the mutant gene. As the mutant gene encodes a major deletion or missense mutation, mice homozygous for the targeted allele do not express the native gene product and can be used to study the effect of a total lack of a given protein. Heterozygotes usually express the protein at levels 50% of normal, allowing the study of the effect of gene dosage. Breeding of various heterozygous and/or homozygous transgenic/knockout animals can be used to combine alterations in the expression of multiple genes and to develop animal models of polygenic diseases (Mauvais-Jarvis and Kahn, 2000).

3. Adenoviral gene transfer technology
A third approach is the adenoviral gene transfer technology. A cDNA sequence to be expressed is introduced into an adenoviral vector. The adenovirus provides an excellent in vivo gene transfer vehicle for efficient hepatocyte transduction and it can be used to introduce the gene efficiently in the liver after systemic administration (Peeters et al., 1996). This technique can be used to overexpress a protein in the liver of a normal mouse, to rescue the function of a deleted gene by liver gene therapy, or to create inducible liver-specific gene knockouts using the Cre-loxP strategy.

4. siRNA technology
Small interference RNA (siRNA) opens a new door to efficiently silence gene expression. It was in 1998 that double stranded RNA (dsRNA) as the mediator of gene silencing was identified in Caenorhabditis elegans and referred to by the term (RNAi) i.e. RNA interference (Fire et al., 1998). Subsequently, an enzyme complex (Dicer) was discovered in Drosophila (Bernstein et al., 2001), as part of a conserved Dicer family expressed in organisms undergoing RNAi (Schütze, 2004). Dicer contains domains for dsRNA binding, RNA unwinding, and ribonuclease activity, and is associated with additional proteins to drive the cleavage of dsRNA in an ATP-dependent manner (Denli and Hannon, 2003). The resulting
siRNA as part of a multiprotein RNA-inducing silencing complex (RISC) is targeted at the complementary RNA species, which is then cleaved (Schütze, 2004). Using siRNAs, a number of disease-related genes have been targeted highlighting the potential of this gene-silencing approach as a therapeutical platform. Some examples include spinobulbar muscular atrophy (SBMA) (Caplen et al., 2002); specific inhibition of the oncogenic K-RAS V12 expression in human tumor cells by retroviral expressing vectors (Brummelkamp et al., 2002); antiviral strategies like HIV-1 (Lee et al., 2002) and Leukaemia (Wilda et al., 2002).

Cardiovascular disorders
1. Hypertension
   a. Transgenic rats overexpressing the mouse Ren 2 gene (TGR (mRen 2)27)
   An introduction and over-expression of the Ren 2 gene in rats results in a severe inherited form of hypertension which can be lethal in homozygous rats if not treated with ACE inhibitors. The exact mechanism underlying this hypertension is not clear but could be the result of altered renin reactivity (Langheinrich et al., 1996). 70 % of rats survive to five months of age, but before that they develop marked cardiac hypertrophy. Drugs acting through the renin-angiotensin system are effective in this model but no effect is observed with calcium channel or β-blockers (Ohta et al., 1996).

   b. Genetic models of Atrial Natriuretic Peptide (ANP) expression
   ANP is the predominant member of a family of at least three structurally and functionally related peptide hormones that elicits potent and brisk natriuresis and diuresis and reduces arterial blood pressure in humans and in a wide variety of other animal species (Brenner et al., 1990). Several genetically engineered mouse models presenting single life-long alterations in the expression of the individual components of the ANP system have been developed. These can be classified into the following groups:
   i. ANP transgenics like transthyretin-ANP (TTR-ANP) transgenic mice Mouse ANP structural gene consisting of all three exons and ~1.75 kb of the 3’ flanking region is isolated from the BALB/cJ genomic library, fused to the transthyretin promoter from the same source, and introduced into the male pronucleus of fertilized embryos of the C3HeB/FeJ background. The incorporation of the transthyretin promoter targets ectopic constitutive expression of the ANP transgene to the liver, resulting in 8- to 10-fold elevation in basal plasma ANP level. The transgenic animals manifest life-long hypertension (25–30 mmHg) relative to their genetically matched nontransgenic (NT) counterparts (Melo et al., 2000; Steinhelper et al., 1990).

   ii. ANP KO (ANP +/-) mice
   A targeting construct containing the neomycin resistance gene is designed to delete 11 base pairs from exon 2 of the mouse pre-proANP by homologous recombination in embryonic stem cells from mouse strain 129. The resulting chimeric mice harboring the mutation are mated to mice of strain C57BL/6J (B6). Matings between the 129 3 B6 heterozygotes produce homozygous mutant (2/2), heterozygous (1/2), and wild-type (1/1) mice in approximate Mendelian ratios. Plasma ANP and ANP-specific atrial granules are undetectable in the 2/2 mice and reduced in the 1/2 mice as compared to the wild-type mice. The homozygous mutant mice are hypertensive (20–30 mmHg) compared with the wild-type siblings (John et al., 1995; Melo et al., 2000).

   iii. Natriuretic peptide receptor KO s (NPR-A and NPR-C)
   The NPR-A KO model developed (Lopez et al., 1995) uses a neomycin “cassette” to replace a sequence of exon 4 in the NPR-A gene that codes for the extracellular ligand-binding dom-
ain in the receptor. NPR-C-deficient mice deletes a sequence of exon 1 of NPR-C that codes for a 215 amino acid sequence of the ligand binding domain (Matsukawa et al., 1999).

c. Neutral endopeptidase (NEP) knockout mice
NEP-deficient mice phenotypically have hypertension, as NEP is the enzyme involved in the degradation of numerous cardiovascular peptides including angiotensin, bradykinin, endothelin and naturetic peptides (Lu et al., 1997).

d. Nitric oxide synthase (NOS)
eNOS knockout mice have been developed which exhibit hypertension due to absent NO generation (Huang et al., 1995), while its overexpression leads to hypotension (Ohashi et al., 1998).

To summarize, these models of hypertension provide the opportunity to search for new mechanisms and new genes in involved hypertension. Many targets in the cardiovascular system have been explored like the knockout models of NO-synthase and ANF, which give evidence that high blood pressure is not only caused by the addition of certain factors, but can also be caused by removal of protective factors.

2. Renin-Angiotensin system (RAS) and transgenic animals
RAS is a circulating hormone system involved mainly in blood pressure and kidney functions. These models address the mechanisms involved in cardiac hypertrophy (Bader, 2002; Nyui et al., 1997). Mouse knockouts have been developed, like the angiotensinogen or angiotensin (AT) receptor knockouts, which eventually develop cardiac hypertrophy.

a. Angiotensinogen
Transgenic mice carrying the rat angiotensinogen gene, develop high blood pressure and typical signs of end-organ damage viz. cardiac hypertrophy and renal fibrosis (Kimura et al., 1992). Interestingly, angiotensinogen expression only in the mouse heart results in normotensive animals developing cardiac hypertrophy indicating thereby that local formation of angiotensin II induces cardiac damage independent of elevated blood pressure (Mazzolai et al., 1998).

b. Renin
Humanized rodent models carrying both the human renin and angiotensinogen genes as transgenes become hypertensive (Ganten et al., 1992; Merrill et al., 1996; Sinn et al., 1999). These gain importance for studying the production and action of angiotensin II in tissues which elicit end-organ damage and for testing human renin inhibitors, which cannot be tested in normal rodents because of species specificity. Additionally, as described above, Ren-2 (TGR(mREN2)27) transgenic rats carrying one of the two murine renin genes develop severe hypertension and cardiovascular hypertrophy despite low angiotensin levels. In contrast, mouse knockout strains of Ren-1a and Ren-2 (two renin genes present in mice) have also been developed by separate gene targeting. These demonstrate morphological alterations in the kidney as evidenced by respectively, enhanced circulating prorenin levels (Clark et al., 1997) to low prorenin levels and increased active renin levels (Sharp et al., 1996).

c. Angiotensin converting enzyme (ACE)
Transgenic rats with ACE overexpression in the heart have been produced which exhibit hypertrophic response only under pressure overload conditions (Tian et al., 1996). A role of ACE in the reproductive system has been demonstrated with ACE knockout mice showing infertile phenotype (Krege et al., 1995).

d. Angiotensin receptors
Different phenotypes have been produced in transgenic animal models using α-myosin heavy chain promoter that overexpress AT1 receptors in the heart. The mouse transgenics developed hypertrophy with high mortality (Paradis et al., 2000), while the rat transgenics developed hypertrophy after
pressure overload (Hoffmann et al., 1996). Further, a role of AT1 receptor (AT1A gene isoform) in cardiovascular regulation is revealed by gene knockout studies (Chen et al., 1997) with the mice being significantly hypotensive.

3. Kallikrein-Kinin system
Kinin peptides exert multiple effects through B1 and B2 receptors on the cardiovascular system. Transgenic animals that overexpress tissue kallikrein or the B2 receptors become hypotensive (Wang et al., 1997), while B2 knockout mice develop salt-sensitive hypertension (Borkowski et al., 1995). On the contrary tissue kallikrein (Meneton et al., 1999) or the B2 receptors knockout mice (Emanueli et al., 1999) develop dilated cardiomyopathy with advancing age. Interestingly, based on the above observations one may conclude that tissue kallikrein nullifies the deleterious effects of angiotensin II on the heart. This is further endorsed by the findings that the beneficial effects of ACE inhibitors in cardiovascular diseases stem from inhibitory effects on the generation of angiotensin II and/or by blocking bradykinin degradation (Bader et al., 2000).

4. Endothelin system
The endothelin system plays an important role in maintaining homeostasis of the circulatory system and in the pathogenesis of cardiovascular diseases. Overexpression of ET-1 and ET-2 in rats and mice leads to normotensive animals with signs of glomerulosclerosis progressing to end-stage renal disease (Hocher et al., 1996). Receptor knockouts ET +/- show elevated blood pressure while the ET/- lead to a lethal phenotype with craniofacial abnormalities (Kurihara et al., 1994). ET$_A$/ET$_B$ double homozygous knockout (ET$_A}$/-$/$ET$_B}$/-) show 100% embryonic lethality due to cardiac failure (Yanagisawa et al., 1998).

5. Adrenoceptor transgenics and knockouts
i. α-adrenoceptor
Mice with altered α-adrenergic receptor (AR) genes have become important tools in elucidating the subtype specific functions of the three α-AR subtypes regulating cardiovascular and neurological functions because of the lack of sufficiently subtype-selective pharmacological agents. Mice with a deletion (knockout, KO) or an overexpression (transgenic, TG) of the α$_{1A}$, α$_{1B}$, or α$_{1D}$-AR subtypes have been generated (Tanoue et al., 2002). Moreover double knockout mice like α$_{1A}$/α$_{1B}$ and the α$_{1B}$AR genes (α$_{1AB}$-KO) or lacking both the α$_{1B}$ and the α$_{1D}$-AR genes (α$_{1BD}$-KO), have been produced (O’Connell et al., 2000). Alternatively, transgenic techniques have also been used to regulate the expression of α$_1$-ARs. Several strains of mice that over express α$_{1B}$-AR have been generated under the control of the myosin heavy chain (MHC) promoter or its homologous promoter (Milano et al., 1994; Zuscik et al., 2000).

α$_2$-adrenergic receptors are implicated in diverse physiological functions particularly of the cardiovascular system and the central nervous system. α$_2$-adrenergic receptor agonists are used clinically in the treatment of hypertension, glaucoma, and attention-deficit disorder, in the suppression of opiate withdrawal, and as adjuncts to general anesthesia (Kable et al., 2000). Mice with a deletion of the α$_{2A}$- (α$_{2A}$-knockout (KO)), α$_{2B}$ - (α$_{2B}$ -KO), or α$_{2C}$ -gene (α$_{2C}$ -KO) have been generated (Altman et al., 1999; Link et al., 1996). More recently, the double knockout mice (α$_{2AC}$ -KO), in which both the α$_{2A}$ - and the α$_{2C}$-genes have been deleted, have been produced (Hein et al., 1999). Mice have also been developed with a point mutation of the α$_{2A}$ –gene (α$_{2A}$ -D79N) (Macmillan et al., 1996).

b. β-adrenoceptor
B-AR subtypes in vivo remain as distinct therapeutical targets due to a number of factors that actually serve to distinguish them. These distinctions include tissue-specific expression patterns, the ability to couple to different G-proteins, pharmacological heterogeneity, and differences in agonist-dependent desensitization (Rohrer et al., 1999). Gene disruption, or “knockout” experiments, has proved to be a useful approach in defining adrenergic receptor
function in vivo. As described above, this technique has been used to disrupt expression of all three α2-AR subtypes, the α2B-AR, and now β1-, and the β3-ARs and most recently, the β2-AR (Rohrer et al., 1999). When the pharmacologic tools outlined above are used in conjunction with genetic techniques, the power to reveal novel functions and mechanisms of action can be greatly enhanced. Mice lacking β1- and/or β2- ARs represent useful model systems for the study of β-AR modulated function in vivo, as well as the role that β-ARs play in pathophysiology. β-adrenoceptors desensitization is mediated through protein kinase A (PKA) and β-adrenoceptor kinase (βARK). Transgenic mice with overexpression of βARK inhibitor exhibit enhanced cardiac contractility while that of βARK1 attenuated isoproterenol-stimulated left ventricular contractility thus demonstrating an important role of βARK in modulating cardiac functions (Koch et al., 1995).

6. Cerebral Stroke
   a. COX-2 transgenics
   Increases in COX-2 enzymatic activity and prostaglandin production have been associated with neuronal injury in both acute and age-related degenerative neurological diseases. COX-2 is constitutively expressed selectively in neurons of the striatum, cerebral cortex, and hippocampus. These COX-2 transgenic mice harbour elevated levels of PGE(2) that are 10-fold higher than nontransgenic levels. A significant increase in infarct volume is observed after middle cerebral artery occlusion with 4 days of reperfusion in COX-2 transgenic mice as compared with nontransgenic littermates (Dore et al., 2003).

   b. XIAP overexpression in neurons
   The X-chromosome linked inhibitor of apoptosis protein (XIAP) is a member of the inhibitor of apoptosis protein (IAP) family and known to inhibit death of various cells under different experimental conditions. Transgenic mice with overexpression of human XIAP in brain neurons have been developed and were shown to be more resistant to brain injury caused by transient forebrain ischaemia after occlusion of the middle cerebral artery compared to control mice. The XIAP transgenic animals exhibited significantly less brain damage, as shown by TUNEL labeling, less reduction in brain protein synthesis, and less active caspase-3 after ischaemia compared with controls (Trapp et al., 2003). Upregulation of RhoB, which is an early indicator of neurological damage, was markedly reduced in the XIAP-overexpressing mice, which had also a better neurological outcome than control animals (Trapp et al., 2003). This together with the increase in XIAP in normal mouse brain in regions surviving the infarct demonstrates that XIAP is an important factor promoting neuronal survival after ischaemia. This could be an exciting target in drug discovery for stroke.

   c. Role of nNOS knockouts in stroke
   Elimination of neuronal nitric oxide synthase (nNOS) by targeted disruption of the nNOS (nNOS -/-) gene results in amelioration of damage seen after hypoxia–ischaemia in the developing brain, since nitric oxide (NO) has been implicated in glutamate-mediated neurotoxicity after ischaemia in cerebral ischaemia models (Ferriero et al., 1996).

7. Congestive heart failure
   a. Spontaneously hypertensive heart failure (SHHF/Mcc-fa1cp) rats
   This is a cross breed of SHR and Koletsky obese rats. These transgenic rats develop early onset hypertension followed by cardiomyopathy and heart failure. Hypertension develops at 3 months of age. Heart failure is more established with advancing age (7, 14, 20 months). This is a good model of dilated cardiomyopathy with hypertension progressing to decompensated heart failure and exhibits several hallmark signs of the human disease state (Anderson et al., 1999; Heyen et al., 2002).

   b. Dilated cardiomyopathy (DCM)
   Murine models relevant to pathogenetic mechanis-
ms in human DCM include overexpression of TNF (Kubota et al., 1997) and conditional cardiac-specific deletion of all VEGF isoforms (Giordano et al., 2001), which may represent an ischemia-mediated mechanism. Mouse models of DCM relevant to abnormal Ca\(^{2+}\) cycling, as seen in human heart failure, include calsequestrin overexpression (Jones et al., 1998) and FKBP 12.6 deficiency with dysfunctional calcium release channels (ryanodine receptors) (Shou et al., 1998). Some of the useful models of DCM include, desmin related myopathy related to missense mutation in the desmin gene (Milner et al., 1996); a naturally occurring genetic DCM in hamsters due to mutation in sarcoglycans gene (SG) leading to deficiency of dystrophin/dystroglycan complex; muscle LIM protein KOs (MLP KO) having a phenotype typical of cardiac failure in humans (Arber et al., 1997) and witch has been used to study the effects of potential therapeutic agents; prolonged overexpression of cardiac adrenergic receptor pathway (≥100 fold) causing late onset DCM (Engelhardt et al., 1999; Liggett et al., 2000); transgenic mice with overexpression of the catalytic subunit of PKA which develop DCM, mild fibrosis and arrhythmias associated with hyperphosphorylation of the ryanodine receptor (RyR2) and phospholamban, without activation of a-AR signaling (Antos et al., 2001) and prolonged overexpression of GTP binding proteins (GTP proteins like G\(_{\alpha}\), G\(_{\beta}\), and G\(_{\gamma}\)) which can induce DCM (Adams et al., 1998; Iwase et al., 1997). Additionally, transgenic mice overexpressing SERCA1 (sarco/endoplasmic reticulum Ca\(^{2+}\) pumps) show enhanced myocardial contractility and increased Ca\(^{2+}\) transport function, while SERCA2 overexpression transgenics exhibit enhanced calcium transients along with accelerated myocardial contractility and relaxation (Loukianov et al., 1998).

c. Lac Z transgenics
Animal transgenics for reporter genes would be useful to follow a given cell lineage during differentiation and regeneration processes. β-galactosidase (lacZ) transgenic rats have been established as a tool for regenerative research (Takahashi et al., 2003). Strong lacZ expression is observed in the skeletal muscles, myocardium, pancreas, and skin obtained from these lacZ-transgenic rats, and moderate lacZ expression was observed in the liver, spleen, kidney, and cartilage (Takahashi et al., 2003). Further, myocardial injury is induced after a lacZ-transgenic bone marrow transplant (BMT) into wild-type rats. This resulted in lacZ-positive cardiomyocytes in the peri-infarct and uninjured myocardium in the BMT recipient rats, suggesting that lacZ-transgenic rats are a useful tool for regenerative research in the myocardium (Takahashi et al., 2003).

8. Thrombosis and haemostasis
Gene knock-outs leading to deficient proteins involved in thrombosis and haemostasis and comparing it with the phenotype-like spontaneous bleeding, platelet defect, prolonged bleeding after surgical trauma etc., has been extremely useful to pinpoint the role played by the particular protein. This can pave the way for designing novel pharmacological agents mimicking the knock-outs, if not lethal (Leadley Jr. et al., 2000). Deletion of FVIII, FIX, vWF and the β-integrin signaling protein of platelet activation result in knock-out mice that closely mirror the human disease states (Bi et al., 1996; Denis et al., 1998; Hodivala-Dilke et al., 1999). Gene knock-outs of factors in the fibrinolytic pathway yield mice with thrombotic susceptibility: viz. plasminogen, tissue plasminogen activator(t-PA), urokinase type plasminogen activator (u-PA) and the combined t-PA/u-PA - resulting in mice that demonstrate impaired fibrinolysis, vascular occlusion and tissue damage due to fibrin deposition (Bugge et al., 1995; Carmeliet et al., 1994; Ploplis et al., 1995).

9. Dyslipidaemia and atherosclerosis
Using genetic manipulation techniques, mice susceptible to atherosclerosis have been created. The peroxisome proliferator-activated receptors (PPARs α-, β- agonists), liver X receptor LXR and
retinoid X receptor RXR ligands show variable efficacy and potency in various models of dyslipidaemia. These include: the inbred strain C57BL6 requiring a 15% fat / 2% cholesterol / 0.5% cholate diet (HF-HC-Cholate diet) and which eventually develop increased LDL and VLDL levels and hypercholesterolaemia (Paigen et al., 1985); transgenics or gene replacement mouse strains APOBh (transgenic for human apolipoproteinB) requiring a HF-HC-Cholate diet and which develop hypercholesterolemia and increased LDL levels (Purcell-Huynh et al., 1995), LDL receptor knockout (KO) mice which are hypercholesterolaemic and develop atherosclerotic lesions after feeding them a Western diet (0.2% cholesterol, 21% fat), LDL receptor KO x APOBh mice which exhibit hypercholesterolaemia and hypertriglyceridaemia along with increased LDL and VLDL levels (Sanan et al., 1998), APOE3 (transgenic for human apolipoprotein E-III) Leiden mice requiring a HF-HC-Cholate diet which and develop hypercholesterolaemia, hypertriglyceridaemia and abnormal ß-migrating forms of VLDL (Groot et al., 1996); Apoe KO (knockout for apolipoprotein E) that develop hypercholesterolaemia, increased VLDL levels after a standard diet (5% fat, <0.05% cholesterol) (Plump et al., 1992); and APOE2KI (mice deficient in murine apolipoprotein E and expressing human apolipoprotein E-II) (Tailleux et al., 2003).

10. Concluding remarks
Reliable murine models resembling many human cardiovascular disorders for hypertension, congestive heart failure, cardiomyopathies, cerebral stroke, thromboembolism and dyslipidaemia have been established. This is important because of the expanding field of gene targeting and gene therapy in molecular cardiology. Differences in response to gene knockouts or overexpression of genes in different genetic mouse strains, poses a major problem in interpreting results obtained with transgenic mice. Novel transgenic technologies like inducible transgene expression and conditional gene targeting for species other than mice like rats and rabbits (Bader et al., 2000) will help to further our knowledge and assist in newer therapeutic strategies.

Acknowledgements
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Table I. Transgenic rodent models for cardiovascular disorders

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<tr>
<th>Disorder</th>
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**Kallikrein-Kinin system**

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**Endothelin system**

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**Adrenergic system**

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<td>Mice</td>
<td>↓ cardiac contractility</td>
<td>(Rokosh and Simpson, 2000)</td>
</tr>
<tr>
<td><strong>Overexpression</strong></td>
<td>Mice</td>
<td>↑ cardiac contractility</td>
<td>(Lin et al., 2001)</td>
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<tr>
<td>α1B-AR</td>
<td>Mice</td>
<td>↓ aortic contractility</td>
<td>(Cavalli et al., 1997)</td>
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<tr>
<td><strong>KO</strong></td>
<td>Cardiac dysfunction</td>
<td></td>
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<tr>
<td>(α MHC promoter/wild type)</td>
<td>Mice</td>
<td>Cardiac hypertrophy, autonomic failure</td>
<td>(Akhter et al., 1997)</td>
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<td>α1D-AR</td>
<td>Mice</td>
<td>hypotension</td>
<td>(Zuscik et al., 2000)</td>
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<tr>
<td><strong>KO</strong></td>
<td>normal aortic contractility</td>
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<tr>
<td>(Isogenic promoter)</td>
<td>Mice</td>
<td>↓ aortic contractility</td>
<td>(Tanoue et al., 2002)</td>
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<tr>
<td>Disorder</td>
<td>Gene manipulation</td>
<td>Strain/Species</td>
<td>Phenotype</td>
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<td>Cerebral Stroke</td>
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<td></td>
<td>α&lt;sub&gt;2A&lt;/sub&gt;-AR KO</td>
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<td></td>
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<td>Mice</td>
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<td>α&lt;sub&gt;2B&lt;/sub&gt;-AR KO</td>
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<td></td>
<td>β&lt;sub&gt;1&lt;/sub&gt; and β&lt;sub&gt;2&lt;/sub&gt; KO</td>
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<td>Mice</td>
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<td>COX-2 enzyme</td>
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<td>Human XIAP overexpression</td>
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<td></td>
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<tr>
<td>Congestive Heart Failure</td>
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<tr>
<td></td>
<td></td>
<td>Rat</td>
<td>Early onset hypertension, cardiomyopathy, heart failure</td>
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<tr>
<td></td>
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<td>Mice</td>
<td>Dilated cardiomyopathy leading to heart failure</td>
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<tr>
<td>Desmin gene missense mutation</td>
<td>Mice</td>
<td>Dilated cardiomyopathy leading to heart failure</td>
<td>(Milner et al., 1996)</td>
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<tr>
<td>Muscle LIM protein KOs</td>
<td>Mice</td>
<td></td>
<td>(Arber et al., 1997)</td>
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<tr>
<td>Adrenergic receptor pathway OE</td>
<td>Mice</td>
<td></td>
<td>(Engelhardt et al., 1999; Liggett et al., 2000)</td>
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<tr>
<td>PKA catalytic subunit OE</td>
<td>Mice</td>
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<td>(Antos et al., 2001)</td>
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<td>GTP binding proteins OE (G, G, G)</td>
<td>Mice</td>
<td></td>
<td>(Adams et al., 1998; Iwase et al., 1997)</td>
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<td>SERCA1 OE</td>
<td>Mice</td>
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<td>(Loukianov et al., 1998)</td>
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<td>LacZ reporter gene OE</td>
<td>Rat</td>
<td>LacZ-positive cardiomyocytes in the peri-infarct and uninjured myocardium</td>
<td>(Takahashi et al., 2003)</td>
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</table>

**Thrombosis and Haemostasis**

<table>
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<th>Disorder</th>
<th>Gene manipulation</th>
<th>Strain/Species</th>
<th>Phenotype</th>
<th>References</th>
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<tbody>
<tr>
<td>FVIII, FIX, vWF and β3-integrin signaling protein KOs</td>
<td>Mice</td>
<td>Spontaneous bleeding, platelet defect, prolonged bleeding after surgical trauma.</td>
<td>(Bi et al., 1996; Denis et al., 1998; Hodivala-Dilke et al., 1999)</td>
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<tr>
<td>Plasminogen, t-PA, u-PA and t-PA/u-PA KOs</td>
<td>Mice</td>
<td>Impaired fibrinolysis, vascular occlusion and tissue damage due to fibrin deposition</td>
<td>(Bugge et al., 1995; Carmeliet et al., 1994; Ploplis et al., 1995)</td>
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**Dyslipidaemia and atherosclerosis**

<table>
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<th>Phenotype</th>
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<tbody>
<tr>
<td>C57BL6</td>
<td>Mice</td>
<td>↑LDL, ↑VLDL and ↑cholesterol</td>
<td>(Paigen et al., 1985)</td>
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<tr>
<td>APOBh</td>
<td>Mice</td>
<td>↑LDL and ↑cholesterol, ↑Cholesterol, ↑triglyceride</td>
<td>(Purcell-Huynh et al., 1995)</td>
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<tr>
<td>LDL KO x APOBh</td>
<td>Mice</td>
<td>↑LDL and VLDL</td>
<td>(Sanan et al., 1998)</td>
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<tr>
<td>APOE3 OE</td>
<td>Mice</td>
<td>↑Cholesterol, ↑triglyceride abnormal VLDL</td>
<td>(Groot et al., 1996)</td>
<td></td>
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<tr>
<td>APOE KO</td>
<td>Mice</td>
<td>↑LDL and ↑cholesterol</td>
<td>(Plump et al., 1992)</td>
<td></td>
</tr>
</tbody>
</table>
Abbreviations: Ren-2, rennin-2; TTR-ANP, transthyretin-atrial natriuretic peptide; NPR, natriuretic peptide receptor; NEP, Neutral endopeptidase; eNOS, endothelial nitric oxide synthase; ACE, angiotensin converting enzyme; AT1/AT1B, angiotensin receptor subtypes; ET1/ET2, endothelin receptor subtypes; KO, knockout; AR, adrenoceptor; COX, cyclooxygenase; XIAP, X-chromosome linked inhibitor of apoptosis protein; SHHF, Spontaneously hypertensive heart failure rats; Lac Z, β-galactosidase; TNF, tumor necrosis factor; SERCA sarco/endoplasmic reticulum Ca\(^{2+}\) pumps; t-PA, tissue plasminogen activator; APOB/E, apolipoprotein B and E.
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