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

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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.

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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 b-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 ß-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 spinobulbular 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 ß-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 hypotension (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-1^{d} 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 knockouts (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_1-adrenergic receptor (AR) genes have become important tools in elucidating the subtype specific functions of the three α_1-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}-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. ß-adrenoceptor

ß-AR subtypes *in vivo* remain as distinct therapeutic 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 α_2-AR subtypes, the α_{1B} -AR, and now $ß_1$-, and the $ß_3$-ARs and most recently, the $ß_2$-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 $ß_1$- and/or $ß_2$- ARs represent useful model systems for the study of ß-AR modulated function in vivo, as well as the role that ß-ARs play in pathophysiology. ß-adrenoceptors desensitization is mediated through protein kinase A (PKA) and ß-adrenoceptor kinase (ßARK). Transgenic mice with overexpression of ßARK inhibitor exhibit enhanced cardiac contractility while that of ßARK1 attenuated isoproterenol-stimulated left ventricular contractility thus demonstrating an important role of ßARK 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-fa^{cp}) 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 calseqestrin 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_s, G_i, and G_q) 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. ß-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 $ß_3$-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-, ß- agonists), liver X receptor LXR and

retinoid X receptor RXR ligands show variable efficacy and potency in various smodels 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 *APOE2*KI (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.

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Table I. Transgenic rodent models for cardiovascular disorders

Disorder	Gene manipulation	Strain/Species	Phenotype	References
Hyper/hypotension				
	Ren-2 gene overexpression	*{TGR (mRen 2)27}* mice	Marked hypertension and cardiac hypertrophy	*(Langheinrich et al.*, 1996; *Ohta et al.*, 1996)
	ANP gene fused to transthyretin promoter	TTR-ANP mice	Life-long hypotension	*(Melo et al.*, 2000; *Steinhelper et al.*, 1990)
	ANP -/-	mice	Hypertensive	*(John et al.*, 1995; *Melo et al.*, 2000)
	NPR-A and NPR-C gene knockouts	mice	Salt diet sensitive hypertension (NPR-A); mild hypotension with bone deformities (NPR-C)	*(Lopez et al.*, 1995; *Matsukawa et al.*, 1999)
	NEP-KO	mice	Hypertension	*(Lu et al.*, 1997)
	eNOS *KO*	mice	Hypertension	*(Huang et al.*, 1995)
	overexpression		Hypotension	*(Ohashi et al.*, 1998)
	Rat angiotensinogen gene KO	mice	Hypotension, kidney dysfunction	*(Kim et al.*, 1995)
	overexpression		Hypertension, end-organ damage	*(Kimura et al.*, 1992; *Mazzolai et al.*, 1998)
	Humn renin angiotensinogen gene overexpression	Rats and mice Mice	Hypertension and end-organ damage	*(Ganten et al.*, 1992; *Merrill et al.*, 1996; *Sinn et al.*, 1999)
	Ren-1^{d} and Ren-2 gene KO	Mice	Hypertension (Ren-1^{d})	*(Clark et al.*, 1997; *Sharp et al.*, 1996)
	overexpression		Hypertension and end-organ damage (Ren2)	*(Veniant et al.*, 1996)
	ACE KO	Mice	Infertility	*(Krege et al.*, 1995)

Disorder	Gene manipulation	Strain/Species	Phenotype	References
	Overexpression AT1/AT1B		Cardiac hypertrophy in pressure overload	*(Tian et al.*, 1996)
	KO	Mice	Hypotension (AT1B)	*(Chen et al.*, 1997)
	Overexpression		Cardiac hypertrophy (AT1)	*(Paradis et al.*, 2000)
Kallikrein-Kinin system				
	tissue kallikrein /B2 receptors *KO*		Dilated cardiomyopathy	*(Emanueli et al.*, 1999; *Meneton et al.*, 1999)
	Overexpression	Mice	Hypotension	*(Borkowski et al.*, 1995; *Wang et al.*, 1997)
Endothelin system				
	ET1/ET2 (ET+/-;ET-/-) *KO*	Mice	Hypertensive, gross craniofacial abnormalities	*(Kurihara et al.*, 1994)
	Overexpression		Normotensive and signs of glomerulosclerosis	*(Hocher et al.*, 1996)
Adrenergic system				
a-adrenoceptors(AR)				
	α_{1A}-AR KO	Mice	↓ cardiac contractility	*(Rokosh and Simpson,* 2000)
	Overexpression (α MHC promoter/wild type)	Mice	↑ cardiac contractility	*(Lin et al.*, 2001)
	α_{1B}-AR *KO*		↓ aortic contractility Cardiac dysfunction	*(Cavalli et al.*, 1997)
	Overexpression (α MHC promoter/wild type) Isogenic promoter	Mice	Cardiac hypertrophy, autonomic failure,	*(Akhter et al.*, 1997)
	α_{1D}-AR *KO*		hypotension	*(Zuscik et al.*, 2000)
	Overexpression		↓ aortic contractility normal aortic contractility	*(Tanoue et al.*, 2002)

Disorder	Gene manipulation	Strain/Species	Phenotype	References
	α_{2A}-AR KO		α_{2A} agonist induced hypotension abolished with ↓ bradycardic effects, ↑ resting heart rate, ↓ presynaptic inhibition of norepinephrine	
	α_{2B}-AR *KO*	Mice	increased α_{2A} agonist induced hypotension while hypertensive effect	*(Kable et al.*, 2000)
	α_{2C}-AR *KO*		abolished ↓ presynaptic inhibition of norepinephrine in α_{2C} -/-	
ß-adrenoceptors				
	ß1 and ß2 *KO*	Mice	Minimal effect on basal heart rate and blood pressure, striking difference between these KOs and wild strains following ß-agonist stimulation or stresses of exercise	*(Rohrer et al.*, 1999)
Cerebral Stroke				
	COX-2 enzyme	Mice	↑ PGE(2) levels and infarct volume	*(Dore et al.*, 2003)
	Human XIAP overexpression	Mice	Smaller brain damage, less reduction in brain protein synthesis and ↓ RhoB upregulation	*(Trapp et al.*, 2003)
Congestive Heart Failure				
	SHHF/Mcc-fa^{cp}	Rat	Early onset hypertension, cardiomyopathy, heart failure	*(Mantero et al.*, 1983; *Okakomoto Aoki*, 1963; *Sustarsik et al.*, 1981)
	Dilated cardiomyopathy TNF OE	Mice	Dilated cardiomyopathy leading to heart failure	*(Kubota et al.*, 1997)
	Conditional cardiac-specific deletion of VEGF isoforms			*(Giordano et al.*, 2001)
	Calsequestrin OE and FKBP 12.6 deficiency	Mice		*(Jones et al.*, 1998; *Shou et al.*, 1998)

Disorder	Gene manipulation	Strain/Species	Phenotype	References
	Desmin gene missense mutation	Mice	Dilated cardiomyopathy leading to heart failure	*(Milner et al.*, 1996)
	Muscle LIM protein KOs	Mice		*(Arber et al.*, 1997)
	Adrenergic receptor pathway OE	Mice		*(Engelhardt et al.*, 1999; *Liggett et al.*, 2000)
	PKA catalytic subunit OE	Mice		*(Antos et al.*, 2001)
	GTP binding proteins OE (G_s, G_i, G_q)	Mice		*(Adams et al.*, 1998; *Iwase et al.*, 1997)
	SERCA1 OE	Mice		*(Loukianov et al.*, 1998)
	LacZ reporter gene OE	Rat	LacZ-positive cardiomyocytes in the peri-infarct and uninjured myocardium	*(Takahashi et al.*, 2003)
Thrombosis and Haemostasis				
	FVIII, FIX, vWF and ß3-integrin signaling protein KOs	Mice	Spontaneous bleeding, platelet defect, prolonged bleeding after surgical trauma.	*(Bi et al.*, 1996; *Denis et al.*, 1998; *Hodivala-Dilke et al.*, 1999)
	Plasminogen, t-PA, u-PA and t-PA/u-PA KOs	Mice	Impaired fibrinolysis, vascular occlusion and tissue damage due to fibrin deposition	*(Bugge et al.*, 1995; *Carmeliet et al*., 1994; *Ploplis et al.*, 1995)
Dyslipidaemia and atherosclerosis				
	C57BL6	Mice	↑LDL, ↑VLDL and ↑cholesterol	*(Paigen et al.*, 1985)
	APOBh	Mice	↑LDL and ↑cholesterol ↑Cholesterol, ↑triglyceride	*(Purcell-Huynh et al.*, 1995)
	LDL KO x APOBh	Mice	↑LDL and VLDL	*(Sanan et al.*, 1998)
	APOE3 OE	Mice	↑Cholesterol, ↑triglyceride abnormal VLDL	*(Groot et al.*, 1996)
	APOE KO	Mice	↑LDL and ↑cholesterol	*(Plump et al.*, 1992)

Abbreviations: Ren-2,rennin-2; TTR-ANP, transthyretin-atrial natiuretic peptide; NPR, natiuretic peptide receptor; NEP, Neutral endopeptidase; eNOS, endothelial nitric oxide syntahse; 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, ß-galactosidase; TNF, tumor necrosis factor; SERCA sarco/endoplasmic reticulum Ca^{2+} pumps; t-PA, tissue plasminogen activator; APOB/E, apolipoprotein B and E.

References

Adams, J.W., Y. Sakata, M.G. Davis, V.P. Sah, Y. Wang, S.B. Liggett, K.R. Chien, J.H. Brown & G.W. Dom: Enhanced Gaq signaling: A common pathway mediates cardiac hypertrophy and apoptotic heart failure. Proc Natl Acad Sci USA 1998, 95 (*17*), 10140 – 10145.

Akhter, S.A., C.A. Milano, K.F. Shotwell, M.C. Cho, H.A. Rockman, R.J. Lefkowitz & W.J. Koch: Transgenic mice with cardiac overexpression of alpha1B-adrenergic receptors. In vivo alpha1-adrenergic receptor-mediated regulation of beta-adrenergic signaling. J Biol Chem 1997, *272*, 21253–9.

Altman, J.D., A.U. Trendelenburg, L. Macmillan, D. Bernstein, L. Limbird, K. Starke, B.K. Kobilka & L. Hein: Abnormal regulation of the sympathetic nervous system in a_{2A}-adrenergic receptor knockout mice. Mol Pharmacol. 1999, *56*, 154–161.

Anderson, K.M., A.D. Eckhart, R.N. Willette & W.J. Koch: The myocardial b-adrenergic system in spontaneously hypertensive heart failure rats. Hypertension 1999, *33* (*part II*), 402-407.

Antos, C.L., N. Frey, S.O. Marx, S. Reiken, M. Gaburjakova, J.A. Richardson, A.R. Marks & E.N. Olson: Dilated cardiomyopathy and sudden death resulting from constitutive activation of protein kinase A. Circ Res 2001, *89*, 997 –1004.

Arber, S., J.J. Hunter, J.J. Ross, M. Hongo, J. Borg, J.C. Perriard, K.R. Chien & P. Caroni: MLP-deficient mice exhibit a disruption of cardiac cytoarchitecutural organization, dilated cardiomyopathy, and heart failure. Cell. 1997, *88*, 393 - 403.

Bader, M: Cardiac Renin-Angiotensin Aldosterone System: Role of the Local Renin-angiotensin System in Cardiac Damage: a Minireview Focussing on Transgenic Animal Models. J Mol Cell Cardiol. 2002, *34*, 1455-1462.

Bader, M., H. Bohnemeier, F.S. Zollmann, O.E. Lockley-Jones & D. Ganten: Transgenic animals in cardiovascular disease research. Exp Physiol. 2000, *85* (*6*), 713-731.

Bernstein, E., A.M. Denli & G.J. Hannon: The rest is silence. RNA 2001, *7*, 1509–1521.

Bi, L., R. Sarkar, T. Naas, A.M. Lawler, J. Pain, S.L. Shumaker & V. Bedian: Further characterization of factor VIII-deficient mice created by gene targeting:RNA and protein studies. Blood. 1996, *88*, 3446-3450.

Borkowski, J.A., R.W. Ransom, G.R. Seabrook, M. Trumbauer, H. Chen, R.G. Hill, C.D. Strader & J.F. Hess: Targeted disruption of a B2 bradykinin receptor gene in mice eliminates bradykinin action in smooth muscle and neurons. J Biol Chem. 1995, *270*, 13706-13710.

Bradley, A., M. Evans, M.H. Kaufman & E. Robertson: Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. Nature 1984, *309*, 255-266.

Brenner, B.M., B.J. Ballermann, M.E. Gunning & M.L. Zeidel: Diverse biological actions of atrial natriuretic peptide. Physiol Rev. 1990, *70*, 665–699.

Brummelkamp, T.R., R. Bernards & R. Agami: Stable suppression of tumorigenicity by virus-mediated RNA interference. Cancer Cell. 2002, *2,* 243–247.

Bugge, T.H., T.T. Suh, M.J. Flick, C.C. Daugherty, J. Romer, V. Solberg, V. Ellis, K. Dano & J.L. Degen: The receptor for urokinase-type plasminogen activator is not essential for mouse development or fertility. J Biol Chem. 1995, *270*, 16886-16894.

Caplen, N.J., J.P. Taylor, V.S. Statham, F. Tanaka, A. Fire & R.A. Morgan: Rescue of polyglutamine-mediated cytotoxicity by double-stranded RNA-mediated RNA interference. Hum Mol Genet. 2002, *11*, 175-184.

Carmeliet, P., L. Schoonjans, L. Kieckens, B. Ream, J. Degen, R. Bronson, R. De Vos, J.J. van den Oord & D. Collen: Physiological consequences of loss of plasminogen activator gene function in mice. Nature. 1994, *368,* 419-424.

Cavalli, A., A.L. Lattion, E. Hummler, M. Nenniger, T. Pedrazzini, J.F. Aubert, M.C. Michel, M. Yang, G. Lembo & C.E.A. Vecchione: Decreased blood pressure response in mice deficient of the a1b-adrenergic receptor. Proc Natl Acad Sci USA. 1997, *94*, 11589–94.

Chen, X., W. Li, H. Yoshida, S. Tsuchida, H. Nishimura, F. Takemoto, S. Okubo, A. Fogo, T. Matsusaka & I. Ichikawa: Targeting deletion of angiotensin type 1B receptor gene in the mouse. Am J Physiol 1997, *272*, F299-304.

Clark, A.F., M.G.F. Sharp, S.D. Morley, S. Fleming, J. Peters & J.J. Mullins: Renin-1 is essential for normal renal juxtaglomerular cell granulation and macula densa morphology. J Biol Chem. 1997, 272, 18185-18190.

Denis, C., N. Methia, P.S. Frenette, H. Rayburn, M. Ullman-Cullere, R.O. Hynes & D.D. Wagner: A mouse model of severe von Willebrand disease:defects in hemostasis and thrombosis. Proc Natl Acad Sci USA. 1998, *95*, 9524-9529.

Denli, A.D. & G.J. Hannon: RNAi: an ever-growing puzzle. Trends Biochem Sci. 2003, *28*, 196–201.

Dore, S., T. Otsuka, T. Mito, N. Sugo, T. Hand, L. Wu, P.D. Hurn, R.J. Traystman & K. Andreasson: Neuronal overexpression of cyclooxygenase-2 increases cerebral infarction. Ann Neurol. 2003, *54* (*2*), 155-162.

Emanueli, C., R. Maestri, D. Corradi, R. Marchioni, A. Minasi, M.G. Tozzi, M.B. Salis, S. Straino, M.C. Capogrossi, G. Olivetti & P. Madeddu: Dilated and failing cardiomyopathy in bradykinin B(2) receptor knockout mice. Circulation. 1999, *100*, 2359-2365.

Engelhardt, S., L. Hein, F. Wiesmann & M.J. Lohse: Progressive hypertrophy and heart failure in α_1-adrenergic receptor transgenic mice. Proc Natl Acad Sci USA. 1999, *96*, 7059 – 7064.

Ferriero, D.M., D.M. Holtzman, S.M. Black & R.A. Sheldon: Neonatal mice lacking neuronal nitric oxide synthase are less vulnerable to hypoxic-ischaemic injury. Neurobiol Dis. 1996, *3*, 64-71.

Fire, A., S. Xu, M.K. Montgomery, S.A. Kostas, S.E. Driver & C.C. Mello: Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998, *391*, 806–811.

Ganten, D., J. Wagner, K. Zeh, M. Bader, J.B. Michel, M. Paul, F. Zimmermann, P. Ruf, U. Hilgenfeldt, U. Ganten, M. Kaling, S. Bachmann, A. Fukamizu, J.J. Mullins & K. Murakami: Species specificity of renin kinetics in transgenic rats harboring the human renin and angiotensinogen genes. Proc Natl Acad Sci USA 1992, *89*, 7806-7810.

Giordano, F.J., H.P. Gerber, S.P. Willimas, N. VanBruggen, S. Bunting, P. Ruiz-Lozano, Y. Gu, A.K. Nath, Y. Huang, R. Hickey, N. Dalton, K.L. Peterson, J.J. Ross, K.R. Chien & N. Ferrara: A cardiac myocyte VEGF paracrine pathway is required to maintain cardiac function. Proc Natl Acad Sci USA. 2001, *98* (10), 5780 – 5785.

Gordon, J.W., G.A. Scangos, D.J. Plotkin, J.A. Barbosa & F.H. Ruddle: Genetic transformation of mouse embryos by microinjection of purified DNA. Proc Natl Acad Sci U S A. 1980, *77*, 7380-7384.

Groot, P.H., B.J. Van Vlijmen, G.M. Benson, M.H. Hofker, R. Schiffelers, M. Vidgeon-Hart & L.M. Havekes: Quantitative assessment of aortic atherosclerosis in APOE*3 Leiden transgenic mice and its relationship to serum cholesterol exposure. Arterioscler Thromb Vasc Biol. 1996, *16*, 926-933.

Hein, L., J.D. Altman & B.K. Kobilka: Two functionally distinct α_2-adrenergic receptors regulate sympathetic neurotransmission. Nature. 1999, *402*, 181–184.

Heyen, J.R.R., E.R. Blassi, K. Nikula, R. Rocha, H.A. Daust, G. Frierdich, J.F.V. Vleet, P.D. Ciechi, E.G. McMahon & A.E. Rudolph: Structural, functional, and molecular characterization of the SHHF model of heart failure. Am J Physiol Heart Circ Physiol. 2002, *283*, H1775-H1784.

Hocher, B., L. Liefeldt, C. Thone-Reineke, H.D.

Orzechowski, A. Distler, C. Bauer & M. Paul: Characterization of the renal phenotype of transgenic rats expressing the human endothelin-2 gene. Hypertension. 1996, *28*, 196-201.

Hodivala-Dilke, K.M., K.P. McHugh, D.A. Tsakiris, H. Rayburn, D. Crowley, M. Ullman-Cullere, F.P. Ross, B.S. Coller, S. Teitelbaum & R.O. Hynes: b3-integrin-deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. J Clin Invest. 1999, *103*, 229-238.

Hoffmann, S., T. Krause, Y. Pinto, H. Buikema, J. Bohlender, H. Nishimura, T. Inagami, D. Ganten & H. Urata: Cardiac-specific expression of the human AT1 receptor in transgenic rats. Hypertension. 1996, *28*, P76.

Huang, P.L., Z. Huang, H. Mashimo, K.D. Bloch, M.A. Moskowitz, J.A. Bevan & M.C. Fishman: Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature. 1995, *377*, 239-242.

Iwase, M., M. Uechi, D.E. Vatner, K. Asai, R.P. Shannon, R.K. Kudej, T.E. Wagner, D.C. Wight, T.A. Patrick, Y. Ishikawa, C.J. Homcy & S.F. Vatner: Cardiomyopathy induced by cardiac Gs alpha overexpression. Am J Physiol. 1997, *272*, H585– H589.

John, S.W.M., J.H. Krege, P.M. Oliver, J.R. Hagaman, J.B. Hodgin, S.C. Pang, T.G. Flynn & O. Smithies: Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. Science. 1995, *267*, 679–681.

Jones, L.R., Y.J. Suzuki, W. Wang, Y.M. Kobayashi, V. Ramesh, C. Franzini-Armstrong, L. Cleemann & M. Morad: Regulation of Ca2+ signaling in transgenic mouse cardiac myocytes overexpressing calsequestrin1998, J Clin Invest. *101* (*7*), 1385 – 1393.

Kable, J.W., L.C. Murrin & D.B. Bylund: In vivo gene modification elucidates subtype-specific functions of a_2-adrenergic receptors. J Pharmacol Exp Ther. 2000, *293*, 1–7.

Kim, H.S., J.H. Krege, K.D. Kluckman, J.R. Hagaman, J.B. Hodgin, C.F. Best, J.C. Jennette, T.M. Coffman, N. Maeda & O. Smithies: Genetic control of blood pressure and the angiotensinogen locus. Proc Natl Acad Sci USA. 1995, *92*, 2735-2739.

Kimura, S., J.J. Mullins, B. Bunnemann, R. Metzger, U. Hilgenfeldt, F. Zimmermann, H. Jacob, K. Fuxe, D. Ganten & M. Kaling: High blood pressure in transgenic mice carrying the rat angiotensinogen gene. EMBO J. 1992, *11*, 821-827.

Koch, W.J., H.A. Rockman, P. Samama, R.A. Hamilton, R.A. Bond, C.A. Milano & R.J. Lefkowitz: Cardiac function in mice overexpressing the b-adrenergic receptor kinase or a bARK inhibitor. Science. 1995, *268*, 1350-1353.

Krege, J.H., S.W. John, L.L. Langenbach, J.B. Hodgin, J.R. Hagaman, E.S. Bachman, J.C. Jennette, D.A. O'Brien & O. Smithies: Male-female differences in fertility and blood pressure in ACE-deficient mice. Nature. 1995, *375*, 146-148.

Kubota, T., C.F. McTiernan, C.S. Frye, S.E. Slawson, B.H. Lemster, A.P. Korestsky, A.J. Demetris & A.M. Feldman: Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor-alpha. Circ Res. 1997, *81*, 627 – 635.

Kurihara, Y., H. Kurihara, H. Suzuki, T. Kodama, K. Maemura, R. Nagai, H. Oda, T. Kuwaki, W.H. Cao & N. Kamada: Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. Nature. 1994, *368*, 703-710.

Langheinrich, M., M.A. Lee & M. Bohm: The hypertensive Ren 2 transgenic rat TGR (mREN2)27 in hypertension research. Characteristics and functional aspects. Am J Hypertension. 1996, *9,* 506–512.

Leadley Jr., R.J., L. Chi, S.S. Rebello & A. Gagnon: Contribution of in vivo models of thrombosis to the discovery and development of novel antithrombotic agents. J Pharmacol Toxicol Meth. 2000, *43*, 101-116.

Lee, N.S., T. Dohjima, G. Bauer, H. Li, M.J. Li, A. Ehsani, P. Salvaterra & J. Rossi: Expression of

small interfering RNAs targeted against HIV-1 rev transcripts in human cells. Nat Biotechnol. 2002, *20*, 500–505.

Liggett, S.B., N.M. Tepe, J.N. Lorenz, A.M. Canning, T.D. Jantz, S. Mitarai, A. Yatani & G.W. Dom: Early and delayed consequences of beta(2)-adrenergic receptor overexpression in mouse hearts: Critical role for expression level. Circulation. 2000, *101*, 1707 – 1714.

Lin, F., W.A. Owens, S. Chen, M.E. Stevens, S. Kesteven, J.F. Arthur, E.A. Woodcock, M.P. Feneley & R.M. Graham: Targeted a1A-adrenergic receptor overexpression induces enhanced cardiac contractility but not hypertrophy. Circ Res. 2001, *89*, 343–50.

Link, R.E., K. Desai, L. Hein, M.E. Stevens, A. Chruscinski, D. Bernstein, G.S. Barsh & B.K. Kobilka: Cardiovascular regulation in mice lacking α_2-adrenergic receptor subtypes b and c. Science. 1996, *273*, 803–805.

Lopez, M.J., S.K.F. Wong, I. Kishimoto, S. Dubois, V. Mach, J. Friesen, D.L. Garbers & A. Beuve: Salt-resistant hypertension in mice lacking the guanylate cyclase-A receptor for atrial natriuretic peptide. Nature. 1995, *378*, 65–68.

Loukianov, E.J.Y., I.L. Grupp, D.L. Kirkpatrik, D.L. Baker, T. Loukianova, G. Grupp, J. Lytton, R.A. Walsh & M. Periasamy: Enhanced myocardial contractility and increased Ca^{2+} transport function in transgenic hearts expressing the fast -twitch skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPase. Circ Res. 1998, *83*, 889-897.

Lu, B., M. Figini, C. Emanueli, P. Geppetti, E.F. Grady, N.P. Gerard, J. Ansell, D.G. Payan, C. Gerard & N. Bunnett: The control of microvascular permeability and blood pressure by neutral endopeptidase. Nat Med. 1997, *3*, 904-907.

Macmillan, L.B., L. Hein, M.S. Smith, M.T. Piascik & L.E. Limbird: Central hypotensive effects of the a_{2a}-adrenergic receptor subtype. Science. 1996, *273*, 801–803.

Mantero, F., G.G. Nussdorfer & C. Robba: Evidence for mineralocorticoid activity in the Milan Hypertensive strain of rats. J Hypertension. 1983, *1,* 150–152.

Matsukawa, N., W.J. Grzesik, N. Takahashi, K.N. Pandey, S. Pang, M. Yamauchi & O. Smithies: The natriuretic peptide clearance receptor locally modulates the physiological effects of natriuretic peptide system. Proc Natl Acad Sci USA. 1999, *96*, 7403–7408.

Mauvais-Jarvis, F. & C.R. Kahn: Understanding the pathogenesis and treatment of insulin resistance and type 2 diabetes mellitus: what can we learn from transgenic and knockout mice?. Diabetes Metab. 2000, *26*, 433-448.

Mazzolai, L., J. Nussberger, J.F. Aubert, D.B. Brunner, G. Gabbiani, H.R. Brunner & T. Pedrazzini: Blood-pressure independent cardiac hypertrophy induced by locally activated renin-angiotensin system. Hypertension. 1998, *31*, 1324-1330.

Melo, L.G., M.E. Steinhelper, S.C. Pang, Y. Tse & U. Ackermann: ANP in regulation of arterial pressure and fluidelectrolyte balance: lessons from genetic mouse models. Physiol Genomics. 2000, *3,* 45–58.

Meneton, P., M. Bloch-Faure, A. Hagege, J.-M. Gasc, W. Huang, M. Neubauer, J. Duffy, J. Menard & F. Alhenc-Gelas: Targeted disruption of the tissue kallikrein gene triggers cardiac abnormalities typical of a dilated cardiomyopathy. Hypertension. 1999, *34*, 33.

Merrill, D.C., M.W. Thompson, C.L. Carney, B.P. Granwehr, G. Schlager, J.E. Robillard & C.D. Sigmund: Chronic hypertension and altered baroreflex responses in transgenic mice containing the human renin and human angiotensinogen genes. J Clin Invest. 1996, *97*, 1047-1055.

Milano, C.A., P.C. Dolber, H.A. Rockman, R.A. Bond, M.E. Venable, L.F. Allen & R.J. Lefkowitz: Myocardial expression of a constitutively active alpha 1B-adrenergic receptor in transgenic mice induces cardiac hypertrophy. Proc Natl Acad Sci USA. 1994, *91*, 10109–13.

Milner, D.J., G. Weitzer, D. Tran, A. Bradley & Y. Capetanaki: Disruption of muscle architecture and myocardial degeneration in mice lacking

desmin. J Biol Chem. 1996, *134*, 1255 – 1270.

Nyui, N., K. Tamura, K. Mizuno, T. Ishigami, K. Hibi, M. Yabana, M. Kihara, A. Fukamizo, H. Ochiai, S. Umemura, K. Murakami, S. Ohno & M. Ishii: Stretch-induced MAP kinase activation in cardiomyocytes of angiotensinogen-deficient mice. Biochem Biophys Res Commun. 1997, *235*, 36-41.

O'Connell, T.D., A. Nakamura, S. Cotecchia, E. Foster & P.C. Simpson: Alpha-1 adrenergic receptors are required for normal postnatal growth of the heart. Circulation. 2000, Suppl Abstract, *II-197*.

Ohashi, Y., S. Kawashima, K. Hirata, T. Yamashita, T. Ishida, N. Inoue, T. Sakoda, H. Kurihara, Y. Yazaki & M. Yokoyama: Hypotension and reduced nitric oxide-elicited vasorelaxation in transgenic mice overexpressing endothelial nitric oxide synthase. J Clin Invest. 1998, *102*, 2061-2071.

Ohta, K., S. Kim & H. Wanibuchi: Contribution of local renin angiotensin system to cardiac hypertrophy, phenotypic modulation and remodeling in TGR (mRen2)27 transgenic rats. Circulation. 1996, *94*, 785–791.

Okakomoto Aoki, K: Development of a strain of spontaneously hypertensive rat. Jap Circ J. 1963, *27*, 282–293.

Paigen, B., A. Morrow, C. Brandon, D. Mitchell & P. Holmes: Variation in susceptibility to atherosclerosis among inbred strains of mice. Atherosclerosis. 1985, *57* (*1*), 65-73.

Paradis, P., N. Dali-Youcef, F.W. Paradis, G. Thibault & M. Nemer: Overexpression of angiotensin II type 1 receptor in cardiomyocytes induces cardiac hypertrophy and remodeling. Proc Natl Acad Sci U S A. 2000, *97*, 931-936.

Peeters, M.J., G.A. Patijn, A. Lieber, P. Meusel & M.A. Kay: Adenovirus-mediated hepatic gene transfer in mice: comparison of intravascular and biliary administration. Hum Gene Ther. 1996, *7(14)*, 1693-1699.

Ploplis, V.A., P. Carmeliet, S. Vazirzadeh, I. Van Vlaenderen, L. Moons, E.F. Plow & D. Collen: Effects of disruption of the plasminogen gene on thrombosis, growth and health in mice. Circulation. 1995, *92*, 2585-2593.

Plump, A.S., J.D. Smith, T. Hayek, K. Aalto-Setala, A. Walsh, J.G. Verstuyft, E.M. Rubin & J.L. Breslow: Severe hypercholesterolaemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell. 1992, *71 (12)*, 343-353.

Purcell-Huynh, D.A., R.V.J. Farese, D.F. Johnson, L.M. Flynn, V. Pierotti, D.L. Newland, M.F. Linton, D.A. Sanan & S.G. Young: Transgenic mice expressing high levels of human apolipoprotein B develop severe atherosclerotic lesions in response to a high fat diet. J Clin Invest. 1995, *95(5)*, 2246-57.

Rohrer, D.K., A. Chruscinski, E.H. Schaublei, D. Bernsteini & B.K. Kobilka: Cardiovascular and metabolic alterations in mice lacking both b1- and b2-adrenergic receptors. J Biol Chem. 1999, *274 (24)*, 16701–16708.

Rokosh, D.G. & P.C. Simpson: The alpha1c/a adrenergic receptor contributes to resting vascular tone and is a potent mediator of vasoconstriction in the mouse. Circulation. 2000, Suppl Abstract, *II-13*.

Sanan, D.A., D.L. Newland, R. Tao, S. Marcovina, J. Wang, V. Mooser, R.E. Hammer & H.H. Hobbs: Low density lipoprotein receptor-negative mice expressing human apolipoprotein B-100 develop complex atherosclerotic lesions on a chow diet: no accentuation by apolipoprotein(a). Proc Natl Acad Sci U S A. 1998, *95 (8)*, 4544-4549.

Schütze, N: At the Cutting Edge: siRNA technology. Mol Cell Endocrinol. 2004, *213*, 115–119.

Sharp, M.G., D. Fettes, G. Brooker, A.F. Clark, J. Peters, S. Fleming & J.J. Mullins: Targeted inactivation of the Ren-2 gene in mice. Hypertension. 1996, *28*, 1126-1131.

Shou, W., B. Aghdasi, D.L. Armstrong, Q. Guo, S. Bao, M.J. Charng, L.M. Mathews, M.D. Schneider, S.L. Hamilton & M.M. Matzuk: Cardiac defects and altered ryanodine receptor function in mice lacking FKBP 12. Nature.

1998, *391*, 489 – 492.

Sinn, P.L., D.R. Davis & C.D. Sigmund: Highly regulated cell type restricted expression of human renin in mice containing 14- or 160-kilobase pair P1 phage artificial chromosome transgenes. J Biol Chem. 1999, *274*, 35785-35793.

Steinhelper, M.E., K.L. Cochrane & L.J. Field: Hypotension in transgenic mice expressing atrial natriuretic factor fusion genes. Hypertension. 1990, *16*, 301–307.

Sustarsik, D.L., R.P. McPartland & J.P. Rapp: Developmental patterns of blood pressure and urinary protein, kallikrein and prostaglandin E2 in Dahl salt hypertension susceptible rats. J Lab Clin Med. 1981, *98*, 599–606.

Tailleux, A., G. Torpier, H. Mezdour, J. Fruchart, B. Staels & C. Fiévet: Murine models to investigate Pharmacological compounds acting as ligands of PPARs in dyslipidemia and atherosclerosis. Trends Pharmacol Sci. 2003, *24 (10)*, 530-534.

Takahashi, M., Y. Hakamata, T. Murakami, S. Takeda, T. Kaneko, K. Takeuchi, R. Takahashi, M. Ueda & E. Kobayashi: Establishment of lacZ-transgenic rats: a tool for regenerative research in myocardium. Biochem Biophys Res Commun. 2003, *305*, 904–908.

Tanoue, A., T. Koshimizu & G. Tsujimoto: Transgenic studies of a_1-adrenergic receptor subtype function. Life Sci. 2002, *71*, 2207–2215.

Tian, X.-L., O. Costerousse, H. Urata, W.-M. Franz & M. Paul: A new transgenic rat model overexpressing human angiotensin-converting enzyme in the heart. Hypertension. 1996, *28*, 520.

Trapp, T., L. Korhonen, M. Besselmann, R. Martinez, E.A. Mercer & D. Lindholm: Transgenic mice overexpressing XIAP in neurons show better outcome after transient cerebral ischaemia. Mol Cell Neurosci. 2003, *23*, 302–313.

Veniant, M., J. Menard, P. Bruneval, S. Morley, M.F. Gonzales & J. Mullins: Vascular damage without hypertension in transgenic rats expressing prorenin exclusively in the liver. J Clin Invest. 1996, *98*, 1966-1970.

Wang, D.Z., L. Chao & J. Chao: Hypotension in transgenic mice overexpressing human bradykinin B2 receptor. Hypertension. 1997, *29*, 488-493.

Wilda, M., U. Fuchs, W. Wossmann & A. Borkhardt: Killing of leukemic cells with a BCR/ABL fusion gene by RNA interference (RNAi). Oncogene. 2002, *21*, 5716–5724.

Yanagisawa, H., M. Yanagisawa, R.P. Kapur, J.A. Richardson, S.C. Williams, D.E. Clouthier, D. De Wit, N. Emoto & R.E. Hammer: Dual genetic pathways of endothelin mediated intercellular signalling revealed by targeted disruption of endothelin converting enzyme-1 gene. Development. 1998, *125*, 825-836.

Zuscik, M.J., S. Sand, S.A. Ross, D.J.J. Waugh, R.J. Gaivin, D. Morilak & D.M. Perez: Overexpression of the a1b-Adrenergic recep tor causes apoptotic neurodegeneration: a multiple system atrophy. Nat Med. 2000, *6*, 1388–94.