HHS Public Access

Author manuscript

J Cardiovasc Transl Res. Author manuscript; available in PMC 2016 June 01.

Published in final edited form as:

J Cardiovasc Transl Res. 2015 June ; 8(4): 269–277. doi:10.1007/s12265-015-9626-4.

2015 Guidelines for Establishing Genetically Modified Rat Models for Cardiovascular Research

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Abstract

The rat has long been a key physiological model for cardiovascular research; most of the inbred strains having been previously selected for susceptibility or resistance to various cardiovascular diseases (CVD). These CVD rat models offer a physiologically relevant background on which candidates of human CVD can be tested in a more clinically translatable experimental setting. However, a diverse toolbox for genetically modifying the rat genome to test molecular mechanisms has only recently become available. Here, we provide a high-level description of several strategies for developing genetically modified rat models of CVD.

Introduction

The rat remains a primary model for cardiovascular disease (CVD) [1] and new technologies for genetically modifying rats are rapidly evolving to include transgenesis [2, 3], gene-trapping [4, 5], gene-targeting [6–9], conditional alleles [10], gene-reporters [11], seamless gene-editing [12], and embryonic stem cell technology [13]. While most of these genetic tools have long been available in the mouse, many just became possible in the rat within the past 5 years (excluding transgenic rats first developed in 1990 [3]). The technical aspects of manipulating the rat genome have been detailed elsewhere [7–9, 14–16]. The goal of this review is to provide a guideline for designing models in the context of CVD research, which is based both on our prior experience [6, 17–19] and observations reported in the literature. We will cover three basic steps in model design: *(1)* identifying the strain background to

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Compliance with Ethical Standards

This article does not contain any studies with animals or human participants performed by any of the authors.

Conflict of Interest: The authors have no personal conflicts of interest to disclose. The Medical College of Wisconsin could one day receive royalties on sales of genetically modified rat strains through a license agreement with Sigma Advanced Genetic Engineering (SAGE).

modify using phenotypic profiles, *(2)* identifying the allele to introduce using genomic and transcriptomic information, and *(3)* choosing and implementing the methodology for introducing the allele. Collectively, we hope that the information below will provide a foundation for any researcher to begin designing and developing new genetically modified models for studying CVD.

Identifying the Strain Background to Modify Using Phenotypic Profiles

Early rat strains were developed primarily through selective breeding for CVD phenotypes and many of these rat strains are still primary models for CVD research [1, 20]. Table 1 highlights the CVD-related phenotypes of commonly used rat strains, which can be used to interrogate a gene-of-interest (GOI) in the context of different models of CVD. Additional phenotyping data can be queried using several tools (e.g., PhenoMiner, Disease Portals, RatMine) in the Rat Genome Database (RGD; http://rgd.mcw.edu) [21]. Phenotype data are standardized and integrated using multiple ontologies, including those for strain background, clinical measurement, measurement method, and experimental conditions and the physiological or pathophysiological traits-of-interest [22]. The “how to” guides for using these and other annotation tools are provided on the RGD website and are highlighted in [21] and [22].

Two large scale rat phenotyping projects have been completed in the past decade, providing broad phenotypic characterization of many inbred rat strains under CVD-relevant conditions [23–27]. The PhysGen Program for Genomic Applications measured more than 200 phenotypes in eleven commonly used rat strains and two complete consomic rat panels (http://pga.mcw.edu/). The National BioResource Project-Rat (NBRP-Rat; http://www.anim.med.kyoto-u.ac.jp/nbr/) in Japan was established to systematically phenotype and cyopreserve well characterized rat strains. To date, the NBRP-Rat has measured 109 phenotypes in >100 rat strains, which can be directly accessed from their data portal [23]. Phenotypic data from both of these programs have been incorporated into RGD’s PhenoMiner database (http://rgd.mcw.edu/phenotypes/) using multiple ontologies in conjunction with specific experimental information and data. In addition, phenotype data from published QTL and select gene modification papers have also been incorporated into PhenoMiner. The PhenoMiner records contain detailed information on *(1)* the study sample, including the strain, sex, age and number of animals used; *(2)* the phenotype, including the clinical measurement, measurement value, standard error and/or standard deviation; *(3)* the method of measurement, including the type of measurement and apparatus used, duration and site of measurement, and the time that the measurement was made post-insult; and *(4)* the experimental conditions under which the measurement that was made, including the type, dose and duration of condition and whether conditions were simultaneous or sequential providing users with a complete view of the experiment [28].

In addition to PhenoMiner, the RGD has developed nine Disease Portals to present users with the genes, QTL, biological processes, and pathways and the rat strains associated with those diseases. The Cardiovascular Disease Portal currently contains 1,432 curated rat genes, 578 QTLs, and 367 strains with cardiovascular annotations. Human and mouse gene and QTL data are also incorporated into each disease portal [29]. Complete genome views

for genes and QTLs annotated by disease association are available for rat, mouse, and human within each disease portal through the RGD GViewer tool (http://rgd.mcw.edu/rgdweb/gTool/Gviewer.jsp). The RGD genome browser (GBrowse; http://rgd.mcw.edu/fgb2/gbrowse/rgd_904/) also allows users to visualize and explore genetic and phenotypic data at the genome level [30]. Within GBrowse, "disease-related tracks" provide an option for viewing genes, QTLs, and strains annotated by disease within a specific genomic region.

Selecting an appropriate phenotypic background for genome modification is one consideration for developing a genetically modified rat model of CVD. If targeting a GOI is expected to increase CVD susceptibility, then one might target this GOI on a naturally resistant background in order to elicit the maximal change in phenotypic effect; and the opposite strategy would be employed when targeting a GOI that is expected to decrease CVD risk. However, this strategy might only be optimal for alleles that exert strong phenotypic effects, whereas a GOI with relatively weak phenotypic effects might be best tested on a CVD-susceptible background, as we have found [17]. Another consideration is that complex CVD is multifactorial – i.e., caused by a combination of multiple genetic, environmental, and lifestyle factors. Thus, a strain's inherent genetic risk to a complex CVD trait (Table 1) or susceptibility to an environmental stressor might be advantageous for testing a GOI in the CVD-relevant context of a particular strains' phenotypic profile.

Identifying the Allele to Introduce Using Genomic and Transcriptomic Information

Selecting a rat strain with a particular allele for a GOI is another consideration prior to genome modification. In some scenarios, the endogenous GOI allele might already be hypomorphic, hypermorphic, or null, in which case one might consider gene-editing, gene KO, or transgenic overexpression to appropriately test the hypothesis. The publically available draft genomes of 39 rat strains on the RGD website (http://rgd.mcw.edu/) [31–33] can be used to predict functionality of endogenous alleles and strain-specific variants (for a detailed review, see [21]). The RGD has two primary tools for variant annotations: the GBrowser (http://rgd.mcw.edu/fgb2/gbrowse/rgd_904) and the Variant Visualizer tool (http://rgd.mcw.edu/rgdweb/front/select.html). To access variant annotations through GBrowser, one simply provides the genomic coordinates or the GOI identifier into the search engine and selects the "Strain Specific Variants" track of GBrowser, which provides variant annotations and PolyPhen functional predictions [34]. The Variant Visualizer tool also provides variant annotations and functional predictions, but in a format that enables comparison of multiple strains simultaneously in tabular form for one or many genes [21]. Using these tools, one can access the allelic variants and their functional predictions for any of the 39 rat strains archived in the RGD, which can be used for informed choice of genetic modification [34].

Expression of a GOI is another consideration for developing a genetically modified rat model of CVD. The most recent *Rattus norvegicus* gene Annotation Release 105 (http://www.ncbi.nlm.nih.gov/genome/annotation_euk/Rattus_norvegicus/105/) was derived from Entrez sequences and 340 RNAseq datasets and has greatly enhanced knowledge of splicing events. However, some genes may still not have accurate models and as such, we

recommend manually aligning the predicted gene products for rat to human and mouse gene products to confirm the annotation before designing the genetic modification. Other tools, including the Rat BodyMap project (http://pgx.fudan.edu.cn/ratbodymap/) [35] and PhenoGen database (http://phenogen.ucdenver.edu/) [36], are publically available to visualize rat transcript expression in multiple tissues. The Rat BodyMap project provides a searchable database to identify transcript expression in 11 organs at four developmental timepoints in both male and female Fischer 344 rats [35]. Similarly, the PhenoGen project includes a searchable database of RNAseq data from 3 organs derived from the BN-Lx and SHR rat strains. For other strain-specific transcript information, there is >1000 rat RNAseq datasets that can be downloaded from the Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) and analyzed by individual researchers.

Choosing and Implementing the Methodology for Genetically Modifying a GOI

Once a GOI and strain have been identified, the tools, methodologies, and resources for rat transgenesis and genome-editing are now available for performing a wide variety of genome modifications in the rat. The first site-directed nucleases, ZFN (zinc-finger nuclease), for targeted gene-editing the need for embryonic stem cells was pioneered in rat in 2010 [6]. Since, two other site-directed nuclease technologies, TALEN (transcription activator-like effector nuclease) [7] and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats) [8, 9], have also been implemented in the rat. Using these technologies the zygotic embryo genome has become accessible for genetic manipulation, thus circumventing many of the limitations of generating novel genetic models through ES cell engineering. This approach has since been adapted and improved for the mouse and many other important research model species and has been heralded as among the top breakthroughs in science for several years running [37–39]. Paired with advances in other transgenic approaches, including transposon-mediated transgenesis [2, 16] and the establishment and genetic manipulation of bona fide germline-competent rat ES cells [11, 40–45], the rat genetic toolbox has improved to the point of making the rat a premier genetic and physiological model for CVD research.

Genome modification using an outbred or inbred strain

Unlike the limited strains of mouse ES cells available, the newly developed genome modification approaches provide equal access to the genomes of any inbred or outbred rat strain, as long as newly fertilized zygotes can be isolated, manipulated, and transferred to generate viable offspring. To date, we have successfully modified 34 different inbred, outbred, consomic, and congenic rat strains spanning a variety of disease areas, including the many of the commonly used models for CVD research.

Outbred strains offer several attractive features for genome modification, including robust breeding, better health, and rapid growth. Additionally, outbred strains offer a CVD-resistant and genetically diverse background on which to test the contributions of alleles with strong effects to CVD (e.g., monogenic CVD candidates). Maintaining the outbred status of a genetically modified strain can require a cumbersome and expensive breeding scheme.

Alternatively, if the outbred-derived transgenic animal is bred to a homozygous state and maintained in a constant sibling intercross breeding pattern, as is often done to reduce breeding costs, then the genetic diversity is completely lost and a specific complement of alleles becomes fixed, creating an entirely new inbred transgenic/mutant rat strain. As a result, this breeding strategy poses experimental challenges in that a genetically suitable wild-type (WT) control does not exist for outbred-derived transgenic/mutant animals, because these individuals (e.g., outbred-derived strain A) are unique to the outbred parental strains and any other individual derived from these strains (e.g., outbred-derived strain B).

Inbred rat strains also offer several advantages and disadvantages for making genetically modified CVD models. Experimentally, the modified strains are easily controlled using the genetically homogenous WT inbred parental strain or WT littermates. However, inbred strains also contain thousands of missense variants, dozens of nonsense variants, and dozens of missing/duplicated genes due to copy number variations [46, 47]. As such, any newly developed inbred strain has a complex architecture of deleterious alleles, which might potentially lead to surprising and sometimes confounding results and difficulties in breeding. At the same time, genetically modified inbred strains also hold several significant advantages for modeling CVD traits. Firstly, most inbred rat strains have been selected for genetic susceptibility to complex CVD traits and therefore provide multiple endogenous variants that are required for pathogenesis of complex diseases, such as hypertension [17, 18]. Secondly, gene-targeting on an inbred background enables interstrain comparisons with other genetically modified individuals derived from the same inbred parental strain, because these individuals are genetically homogenous except for the unique genome modifications. We recently used this strategy to demonstrate the relative effects of multiple causative alleles cosegregating at a single GWAS locus [17].

In sum, the selection of an inbred or outbred genetic background is crucial to the desired phenotype and interpretation of results and should be fully considered at the outset of developing any genetic model and when critically evaluating our peers. Working within the framework of a familiar model must be weighed against the available resources and the potential for conflicting or misinterpreted results. In the end, it is likely a diverse set of inbred and outbred genetic models in a diverse set of genetic backgrounds will be developed and leverage the full knowledgebase of rat cardiovascular physiology and genetics and ultimately further distinguish the rat model from the mouse model for studying complex CVD.

Site-directed nucleases

The mechanisms and various similarities, differences, benefits, and drawbacks of ZFNs, TALENs, and CRISPR/Cas9 and their modifications have been covered in-depth elsewhere [48–50]. As such, we will only highlight the most pertinent points as they pertain to targeting genes in rat embryos. However, it is noteworthy to mention at the outset that most of the non-repetitive rat genome appears to be accessible by at least one or more of these gene-editing technologies.

To distinguish between the site-directed nucleases, we first recognize that all nuclease-based technologies are designed to accomplish the same feat: to direct a double-strand break

(DSB) in the genomic DNA at the investigator-specified location in the rat genome. Once a DSB is induced, the zygotic embryo cell takes over to repair the genome. In the absence of homologous DNA template, the DSB is repaired most frequently by nonhomologous end joining (NHEJ), occasionally resulting in loss or gain of small segments of DNA ranging from ~1–1000bp, with a median of ~10bp of modified genomic sequence flanking or centered on the target site. Within the protein coding DNA sequence (CDS) of a gene, an insertion/deletion (indel) may remove protein coding information (i.e., loss of amino acids) or cause a frameshift mutation, disrupting the open reading frame downstream of that position. In the case where a global gene knockout is desired, most often the cleavage is directed to a coding portion of the gene, preferably within the first 50% of the CDS. When a frame-shifting indel is generated in these regions, the resulting protein product is generally severely truncated to mimic a null allele, or the mRNA may be targeted for nonsense-mediated RNA decay (NMD) and no protein product is generated [48]. Alternatively, if a homologous template is provided, such as a single-stranded oligonucleotide (ssODN) or plasmid DNA containing homology arm sequences that flank the target site, the embryo cell can be used for homology-directed repair (HDR) to repair the genomic DSB. This process can then be used to insert or "knock in" new sequences site-specifically and precisely into the rat genome. At present, NHEJ and HDR events can be catalyzed by ZFN, TALEN, or CRISPR/Cas9 and variations thereof, so the selection of which system to use largely comes down to other factors: design parameters, potential for off-target effects, relative efficiency in the embryo, and cost, as described below.

Target site accessibility, specificity, and efficiency of site-directed nucleases

An important design consideration is the genome-wide accessibility of each nuclease technology. CRISPR/Cas9 interacts preferably with a canonical protospacer-adjacent motif (PAM) that is 5′-NGG-3′ (where N is any nucleotide), a dinucleotide sequence which appears roughly every ~16-bp in the rat genome. In comparison, commercially available ZFN reagents have been touted as being able to target roughly every ~50bp in the rat genome. The TALEN reagents, however, offer perhaps the greatest flexibility, requiring only precise spacing between the monomer binding sites and no other strict sequence requirements.

For precise modification of the genome, the number of base-pairs that are recognized by the site-directed nuclease typically determines the specificity of that target binding site within the genome, which has been reported as follows: CRISPR/Cas9<ZFN<TALEN [50]. While it can be useful and acceptable to bioinformatically predict potential off-target sites and screen founder animals for off-target mutations, one cannot exclude other potential sites in the genome. Unbiased approaches have been developed (mostly in cell culture systems) and several of these studies have guided better reagent designs (reviewed in [50]). At present, the high activity and shorter recognition sequence of the current generation CRISPR/Cas9 system leads to a greater propensity for off-target effects compared to ZFNs and TALENs [50]. However, it is likely that target-site and reagent-specific off-target effects in embryos occur at relatively low-frequency with all three nuclease technologies [51–56], albeit off-target effects for ZFN and TALEN are reported considerably less frequently than CRISPR/Cas9. Of note however, the most recent CRISPR/Cas9 reagents, using a FokI endonuclease

domain fused to a catalytically inactive Cas9 to create a heterodimeric targeting system similar to ZFN and TALEN, appears to dramatically increase specificity in cell models [57], but to our knowledge has yet to be demonstrated in rodent embryos. Ultimately, off-target mutations can be an unwanted consequence of any genome editing approach and, as such, we recommend routinely backcrossing new transgenic/mutant lines for multiple generations to clean the genomic background of any potential off-target mutations.

After considering the accessibility and specificity of each reagent, one might also consider the relative efficiency of each nuclease technology, which ultimately impacts the overall cost of generating a genetically modified strain. Over the past 6 years, we have targeted hundreds of genes across multiple inbred and outbred rat stains (Table 2). We found that CRISPR/Cas9 is by far the most active system in rat embryos, requiring less than 90 embryo injections on average per mutant founder generated, followed by commercially available ZFNs (Table 2). In comparison, TALENs have been considerably less efficient in rat embryos compared to the CRISPR/Cas9 and ZFN in our experience. Thus, although the current CRISP/Cas9 reagents are perhaps the least specific nuclease technology, our data suggest that they have the highest activity and therefore might be the best option for gene-targeting in the rat, as long as backcrossing is used to reduce off-target effects. Efficiency of nuclease reagents can also be screened *in vitro* to predict activity *in vivo*. This protocol entails transfection of plasmid or mRNA encoding the reagents; PCR of the target locus after 48–72 hours and detection of indels using the Cel-I nuclease assay (described in detail in [58]).

Cost, availability, services, and other considerations for site-directed nucleases

In terms of cost, the introduction of TALENs to the market has dramatically reduced the cost of custom ZFN reagents. However, the simplicity and ability for labs to generate their own CRISPR/Cas9 reagents using plasmids readily available from Addgene (https://www.addgene.org/) has led to an extremely rapid uptake of CRISPR/Cas9 approach. With a significant cost advantage, it is likely that the CRISPR/Cas9-based genome editing will dominate the field, especially as improvements are made to increase specificity of this approach.

The ability to share and license the genetically modified animals produced with these approaches is also a potential consideration when designing a model. The details surrounding intellectual property rights remain murky in the fast evolving genome editing field. Following their recent acquisition of SAGE Laboratories, the Horizon Discovery Group owns the exclusive rights for licensing rat models produced using ZFNs and has an exclusive license from Caribou Biosciences, Inc. (http://cariboubio.com/) to license rats produced using CRISPR/Cas9, however, these models can be routinely shared between academic researchers through material transfer agreements. Researchers at the Broad Institute were awarded the first patents on CRISPR/Cas9 technology in 2014 and have widely distributed the technology for producing modified animals without restrictions on academic sharing. Several companies now provide custom genetic engineering services in rat using CRISPR/Cas9. Transposagen Biopharmaceuticals, Inc. (http://www.transposagenbio.com/) has licensed the next-generation CRISPR/Cas9-FokI system

from the Broad Institute for producing rat models and holds exclusive rights to licensing rats produced using the piggyBac transposon technology. To date, multiple separate patents have been issued surrounding TALENs and their applications, but TALEN reagent assembly platforms have been distributed widely within academia and no current restrictions exist on sharing TALEN-generated models.

Targeted insertion and knockin modifications using site-directed nucleases

In addition to gene-targeting, co-injection of exogenous template and nuclease reagents can be used to insert or modify specific genomic sequences by HDR in both rat [10, 59, 60] and mouse [10, 61–63] embryos. In terms of design, the targeted double strand break must be introduced in the immediate vicinity (preferably less than 20bp) from the intended insertion or modification site. Even when providing a template to drive HDR, a cell can still repair the DNA break via both the NHEJ repair mechanisms. Thus, highly active reagents with high cleavage activity are generally necessary to achieve the desired knockin by HDR. These tight design parameters may limit the approach to one or another of the nuclease technologies as described above, depending on the target. Another current barrier to routine knockin via HDR, at least by embryo microinjection, appears to be the size of the inserted fragment, with current reports of successful integration in the 4–5kbp range in mouse embryos [64] and ~1.5kbp Cre and GFP transgenes in rat embryos [59]. This apparent restriction on size likely limits the types of transgenes and reporters that may be homologously integrated into the genome. Despite these limitations, the modification of specific gene alleles and single or multiplex insertion of short sequences, such as loxP sequences to generate loxP-flanked, or “floxed”, conditional gene alleles in rat have now been established by multiple groups [10, 60]. In particular, the ability to create conditional gene alleles in the rat suggests that the long-awaited capability for tissue-specific and temporally regulated gene knockout in the rat is on the near horizon.

Tissue-specific and conditional mutagenesis

A benchmark tool for the gene-editing community has been the ability to induce tissue-specific and conditional gene-targeting. The generation of floxed gene alleles and Cre transgenic models represents a considerable investment of time and resources, since each loxP site requires its own site-directed nuclease and a Cre-transgenic model must also be produced and initially characterized before the models can be bred together and experimental animals generated. However, once a collection of Cre models have been developed, the tools and approaches are likely to proliferate rapidly as strains are shared between laboratories and institutions. Useful transgenic Cre-reporter strains such as the CAG.loxP.EGFP model developed by the laboratory of Dusan Bartsch [65], which expresses the enhanced green fluorescent protein upon Cre-mediated excision, is available from the Rat Resource and Research Center (RRRC) and will facilitate the initial characterization of these Cre models.

Transgenesis approaches

The standard approaches for transgenic overexpression in rats have been established for 25 years and the procedures closely mimic those routinely used to produce transgenic mice [66]. Plasmid-based transgenes and bacterial artificial chromosome (BAC)-based transgenes

are routinely used in rats [10, 67–69] and BACs that are recombineered to carry Cre recombinase transgenes have recently been used to produce some of the first Cre transgenic models in rats [65, 68, 70]. Lentivirus-based transgenesis is another established technique in the rat [71, 72], although this transgenesis method is considerably less prevalent. In contrast, a non-viral transgenic approach using the *Sleeping Beauty* (SB) or *Piggybac* (PB) transposon systems is becoming more routinely used as the preferred method of transgenesis, due to its efficiency [2]. These engineered two-part transposable element systems are highly flexible and simple to use, have the capacity to mobilize large transgenes [73], and result in the insertion of single-copy transgene units at random into the embryo genome which reliably and stably express transgenes for many generations [2]. These single-copy transgene units are also easily positionally cloned to allow the researcher to pinpoint the site of transgene insertion and know that no other genes have been disrupted by the transgene integration.

Embryonic stem cells

With rapidly advancing genome editing tools such as the site-directed nucleases and transposable element technologies which can modify the zygotic embryo genome directly, less attention has been directed toward genetic engineering in rat embryonic stem cells (ESC). However, considering the above limitations in efficiency, size of engineered knockins, and potential for intellectual property issues and scalability, the rat ESC still promises to have a significant and important role in the full potential of the rat model for CVD research. First established in 2008, authentic germline-competent rat ESC have now been derived from a few inbred, outbred, and hybrid strains, including Dark Agouti, Fisher F344, and Sprague Dawley [40, 45, 74–76]. These cell lines are also currently available through the RRRC. To date, a few examples of genome modification via classical homologous gene targeting have been successfully reported [15, 42, 45, 77] and recent studies have deployed site-directed nucleases to dramatically improve the efficiency of gene targeting in ESC to create new models [44]. In addition, recent breakthroughs in transposon-based mutagenesis, and potentially the use of site-directed nucleases, in cultured rat spermatogonial stem cells (SSC) will allow for scalable production of new models [16, 78]. In the future, combining this improved efficiency with the scalability of ESC and SSC modification in culture will provide attractive strategies for rapidly modifying multiple genes and for creating resources of genetically modified rat models as has been so successful in large scale efforts in the mouse.

Conclusions

Manipulation of the rat genome is no longer a considerable weakness of the rat as a model for genetic approaches to CVD research. The unique physiological attributes of genetic rat models of CVD can now be fully exploited using the diverse genetic toolbox described above to generate more clinically tools for studying CVD. Several areas still remain to be fully developed in the rat, namely tissue-specific and conditional alleles. However, the rapid proliferation of new genome editing technologies over the past few years, suggest that these hurdles will eventually be overcome. As genome editing and ESC technology in the rat proliferates, it will be essential for the rat research community to adopt strain nomenclature

guidelines developed for naming transgenic and mutant rat models. Guidelines have been developed by the International Committee on Standardized Genetic Nomenclature for Mice and the Rat Genome and Nomenclature Committee and can be accessed at http://www.informatics.jax.org/mgihome/nomen/gene.shtml. The RGD has developed a strain registration form found at http://rgd.mcw.edu/tools/strains/strainRegistrationIndex.cgi to assist researchers in properly registering these models.

Acknowledgments

Funding: This work was supported in part by R01CA193343 (M.J. Flister), K01ES025435 (J.W. Prokop), and DP2OD008396 (A.M. Geurts).

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Table 1

Disease-susceptible inbred rat strains commonly used for cardiovascular research.

Strain	Designation	Phenotype(s)	Ref (PMID)
Dark Agouti	DA/OlaHsd	Increased aerobic running capacity; Increased cardiac output; increased heart rate; Increased heart weight	12079278
Fawn Hooded Hypertensive	FHH/Eur	Impaired myogenic response; Abnormal kidney physiology; Increased systemic arterial blood pressure; Increased urine protein level, Pulmonary hypertension	9887194, 9184548
Genetically Hypertensive	GH/Omr	Abnormal blood vessel morphology; Hypertension; Increased mean systemic arterial blood pressure; Increased systemic arterial blood pressure	4593562
Goto Kakizaki	GK/KyoSwe	Cortical renal glomerulopathies; Circulating insulin level; Impaired glucose tolerance; Increased body weight; Increased circulating glucose level	8528248
Inherited stress-induced arterial hypertension	ISIAH	Increased circulating corticosterone level; Increased mean systemic arterial blood pressure; Increased response of heart to induced stress; Increased systemic arterial systolic blood pressure	4040537
Lyon hypertensive	LH/MavRRRC	Hyperlipidemia; Increased body weight; Increased systemic arterial blood pressure	9247739
Milan hypertensive strain	MHS/N	Decreased kidney weight; Increased heart rate, Increased left ventricle weight, Increased systemic arterial blood pressure	8846504, 16278339
Milan normotensive strain	MNS	Decreased systemic arterial blood pressure; Glomerulosclerosis	8846504, 16278339
Prague hypertensive/normotensive strains	PHR, PNR	Mild hypertension	8401416, 2264160
Sabra hypertension prone	SBH/Ygl	Increased circulating creatinine level, Increased renal glomerular filtration rate, Increased systemic arterial blood pressure; Increased urine protein level, Salt-sensitive hypertension	8906515
Salt sensitive	SS/Jr	Albuminuria; Cardiac fibrosis; Enlarged heart; Heart left ventricle hypertrophy; Hyperlipidemia; Hypertension; Increased systemic arterial blood pressure; Increased urine protein level; Insulin resistance	6754600
Spontaneoulsy hypertensive rat	SHR	Increased systemic arterial blood pressure; Abnormal cardiac stroke volume; Increased circulating glucose level	13939773, 13939773, 3596765
Spontaneously hypertensive heart failure rats	SHHF/Bbb	Congestive heart failure; Increased systemic arterial systolic blood pressure	18443590
Spontaneously hypertensive rats, stroke prone	SHRSP	Abnormal cardiac stroke volume; Decreased body weight; Hypertension; Increased systemic arterial blood pressure; Stroke; proteinuria; Cardiovascular abnormalities	4454512
Zucker Diabetic Fatty rat	ZDF	Decreased circulating insulin level; Increased circulating glucose level; Obese; Increased susceptibility to autoimmune diseases; Vasculitis	2786848

PMID; Pubmed ID

Table 2

Mutagenesis and transgenesis in rats using ZFNs, TALENs, CRISPRs, and Sleeping Beauty transposons

Number of Gene Targets/Transgenes	Project type	Embryos Injected	Transferred	Liveborn	Screened	Positive Founders	Founder Rate	Project Success
120	ZFN	40,928	22,723	4,242	3,343	564	16.9%	95.8%
24	TALEN	12,773	6,118	1,145	908	33	3.6%	50.0%
32	CRISPR	5,789	3,100	462	322	110	34.2%	90.6%
26	Sleeping Beauty	6,278	3,107	717	587	126	21.5%	92.3%