Published in final edited form as:

Basic Res Cardiol. ; 113(1): 8. doi:10.1007/s00395-017-0666-6.

Gene Expression Analysis to Identify Mechanisms Underlying Heart Failure Susceptibility in Mice and Humans

Christoph Koentges^{1,*}, Mark E. Pepin^{2,*}, Carolyn Müsse¹, Katharina Pfeil¹, Sonia V. Viteri Alvarez¹, Natalie Hoppe¹, Michael M. Hoffmann^{3,4}, Katja E. Odening^{1,3}, Samuel Sossalla⁵, Andreas Zirlik^{1,3}, Lutz Hein^{3,6}, Christoph Bode^{1,3}, Adam R. Wende^{2,#}, and Heiko Bugger^{1,3,#}

¹Cardiology and Angiology I, Heart Center Freiburg University, Freiburg, Germany

²Department of Pathology, Division of Molecular and Cellular Pathology, University of Alabama at Birmingham, Birmingham, AL, USA

³Faculty of Medicine, University of Freiburg, Germany

⁴Institute for Clinical Chemistry and Laboratory Medicine, Medical Center – University of Freiburg, Germany

⁵Department of Internal Medicine II, University Hospital Regensburg, Germany

⁶Institute of Experimental and Clinical Pharmacology, and BIOS Center for Biological Signaling Studies, University of Freiburg, Germany

Abstract

Background—Genetic factors are known to modulate cardiac susceptibility to ventricular hypertrophy and failure. To determine how strain influences the transcriptional response to pressure overload-induced heart failure (HF) and which of these changes accurately reflect the human disease, we analyzed the myocardial transcriptional profile of mouse strains with high (C57BL/6J) and low (129S1/SvImJ) susceptibility for HF development, which we compared to that of human failing hearts.

Methods and Results—Following transverse aortic constriction (TAC), C57BL/6J mice developed overt HF while 129S1/SvImJ did not. Despite a milder aortic constriction, impairment of ejection fraction and ventricular remodeling (dilation, fibrosis) was more pronounced in C57BL/6J mice. Similarly, changes in myocardial gene expression were more robust in C57BL/6J (461 genes) compared to 129S1/SvImJ mice (71 genes). When comparing these patterns to human dilated cardiomyopathy (1,344 genes), C57BL/6J mice tightly grouped to human hearts. Overlay and bioinformatic analysis of the transcriptional profiles of C57BL/6J mice and human failing hearts identified six co-regulated genes (POSTN, CTGF, FN1, LOX, NOX4, TGFB2) with

Corresponding authors: Heiko Bugger, MD PhD, Heart Center Freiburg University, Cardiology and Angiology I, Hugstetter Str. 55, 79106 Freiburg, Germany, Phone: +49 761 34010, Fax: +49 761 270 34412, heiko.bugger@universitaets-herzzentrum.de, Adam R. Wende, PhD, University of Alabama at Birmingham, Department of Pathology, 901 19th Street South, BMR2 Rm 506, Birmingham, AL 35294, United States, Phone: +1 205 975-6272, Fax: +1 205 934-7447, adamwende@uabmc.edu.

*Authors contributed equally

#Corresponding authors contributed equally

Disclosures

The authors declare that they have no conflict of interest.

established link to HF development. Pathway enrichment analysis identified angiotensin and IGF-1 signaling as most enriched putative upstream regulator and pathway, respectively, shared between TAC-induced HF in C57BL/6J mice and in human failing hearts.

Conclusions—TAC-induced heart failure in C57BL/6J mice more closely reflects the gene expression pattern of human dilated cardiomyopathy compared to 129S1/SvImJ mice. Unbiased as well as targeted gene expression and pathway analyses identified periostin, angiotensin signaling, and IGF-1 signaling as potential causes of increased HF susceptibility in C57BL/6J mice and as potentially useful drug targets for HF treatment.

Keywords

heart failure; transverse aortic constriction; gene expression; genetic background; cardiac function

Introduction

Left ventricular (LV) hypertrophy, or increased LV myocardial mass, is associated with many cardiovascular disorders and is recognized as an independent risk factor for cardiac-related morbidity and mortality [18, 36]. Studies on the Framingham population show that, despite adjusting for other heart failure (HF) risk factors, hypertension is the foremost precursor to HF, increasing an individual's risk by 2–3-fold [24, 36]. Interestingly, the cardiac hypertrophic response to chronic hypertension may vary significantly, including concentric hypertrophy, eccentric hypertrophy, and even unchanged LV geometry [16]. LV geometry and LV mass vary considerably among patients with comparable extent of hypertension, suggesting a significant contribution of genetic factors contributing to the development of specific LV geometry. Estimates attribute as much as 60 % of the blood pressure independent variation in cardiac mass to genetic factors [64]. Furthermore, race and ethnic background have been shown to correlate to the relative risk for concentric LV hypertrophy and increased LV mass, also suggesting the presence of genetic modifiers conferring differential susceptibility to pressure overload-induced LV remodeling [7, 28, 41].

A frequently used model to induce chronic LV pressure overload, progressive LV hypertrophy remodeling, and subsequent HF in mice is the murine transverse aortic constriction (TAC) model developed by Rockman et al [53]. This model has been extensively used to elucidate fundamental signaling processes involved in cardiac hypertrophy and HF development [9, 12, 37, 48, 68]. In addition, the TAC model has been routinely used on numerous genetically engineered mice to investigate the role of specific genes during the development of LV hypertrophy and cardiac failure in vivo [2, 9, 33, 37, 39, 51]. Interestingly, Barrick and colleagues reported differential susceptibility for pressure overload-induced hypertrophy in inbred C57BL/6J and 129S1/SvImJ mice which can vary even within substrains, i.e. C57BL/6J [4, 17]. In the former, C57BL/6J mice demonstrated early transition of hypertrophy to heart failure following TAC, whereas 129S1/SvImJ mice showed a delayed transition to heart failure. Thus, the differential susceptibility for development of LV hypertrophy and subsequent heart failure in response to pressure overload observed in humans is mirrored by observations in inbred mice on different genetic backgrounds, supporting the concept that alterations in gene expression may significantly contribute to differential susceptibility for LV hypertrophy and HF in humans. Indeed,

differential susceptibility for various diseases has been attributed to a number of mechanisms affecting gene expression, including genetic heterogeneity, epigenetic regulation, environmental stimuli, incomplete penetrance of alleles, or effects of modifier genes [45].

The molecular mechanisms determining both heart failure susceptibility and the transition from adaptive hypertrophy to decompensated heart failure in humans are incompletely understood. Based on the differential susceptibility for cardiac hypertrophy and failure in different inbred mouse strains, we hypothesized that the differential gene expression patterns in response to pressure overload determines the susceptibility to develop cardiac dysfunction and heart failure. To test this hypothesis, we subjected C57BL/6J and 129S1/SvImJ mice to TAC, which show clear differences in the susceptibility to developing heart failure in response to chronic pressure overload. We also exploited that inbred mouse strains show a very narrow range of phenotypes compared to humans with a naturally high degree of genetic heterogeneity. We aimed to generate hypotheses on which genes and/or pathways may regulate the differential susceptibility to develop heart failure in response to pressure overload using gene expression analysis and subsequent bioinformatic analysis. In addition, the murine transcriptional patterns were compared to those of human failing hearts in order to generate hypotheses regarding genes and/or pathways that may fundamentally contribute to conserved mechanisms governing heart failure development.

Methods

Animals

Male C57BL/6J and 129S1/SvImJ mice were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were housed in individually-ventilated cages with 12 h daylight/dark cycles at 22°C. Animals were fed a laboratory standard chow and had free access to water. The study conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health and was performed after securing approval by the Regierungspräsidium Freiburg (G-16-137).

Human samples

Samples from the left ventricle were obtained either from patients with end stage dilated cardiomyopathy (DCM) that underwent transplantation (Table S1), or from healthy donor hearts that could not be transplanted for technical reasons (n = 5). Samples were snap frozen in liquid nitrogen and stored at -80°C. All procedures were conducted in compliance with the local ethics committee (University of Göttingen, Germany), and written and informed consent was received from all subjects before inclusion.

Transverse aortic constriction (TAC)

Minimally-invasive transverse aortic constriction was performed as published previously [51]. In brief, 8-week-old male C57BL/6J or 129S1/SvImJ mice were anesthetized with a single intraperitoneal injection of ketamine (100 mg/kg) and xylazine (6 mg/kg). Following a suprasternal skin incision, a partial thoracotomy was performed by a 2 mm longitudinal incision of the sternum and a microsurgical clip was applied on the transverse aorta between

the left carotid artery and the brachiocephalic trunk using a micro clip applicator. The thorax was closed using suture, and mice recovered on a warming pad until they were fully awake. To identify the minimum clip diameter to achieve successful induction of heart failure by TAC, mice received clips of different size: C57BL/6J mice received clips with a diameter of 27G, 30G, or 32G. 129S1/SvImJ mice received clips with a diameter of 30G, 32G or 34G. Sham procedures were performed in identical fashion except for clip application. After 10 or 12 weeks, C57BL/6J or 129S1/SvImJ mice were anesthetized using 0.3 mg/g body weight thiopental i.p. and hearts were excised, weighed, and snap frozen for further experiments.

Transthoracic echocardiography

Transthoracic echocardiography was performed as described previously [30]. Mice were anesthetized using isoflurane inhalation (3% for induction and 2% for maintenance of anesthesia). Echocardiography was performed before TAC and every 2 weeks after TAC until sacrifice using a Vivid 7 Dimension (GE Healthcare, Munich, Germany) echocardiograph equipped with an i13L transducer (14 MHz). M-Mode images and 2D parasternal short axis images were taken, and ejection fraction and LV geometry were quantified as described by others [25]. Peak trans-stenotic pressure gradients were measured at the site of constriction using pulsed-wave Doppler three days post surgery.

Histology

Histological analysis was performed in 10 µm cross sections with a distance of 200 µm. The following antibodies were used to estimate angiogenesis, cell proliferation and macrophage infiltration: anti-CD31, anti-Mac 3 (BD Pharmingen, Franklin Lakes, USA), anti-Ki-67 (Abcam, Cambridge, UK), rabbit anti-rat and goat anti-rabbit secondary antibodies (VectorLabs, Burlingame, CA). TdT-mediated dUTP Nick-End Labeling (TUNEL) was performed using a kit system according to the manufacturer's protocol (Promega, Madison, USA). Mean amount of positive signals per three sections were calculated as fold change relative to Sham operated animals. Collagen deposition and cardiomyocyte size were determined using Masson's trichrome staining (Sigma, Weelze, Germany) and wheat germ agglutinin (WGA) staining (Life Technologies, Darmstadt, Germany), respectively [30]. The area of fibrosis was determined in 4 randomly chosen sections with 4 randomly chosen areas per section, while blindly moving the microscope at 20× magnification. A grid was placed over the image, followed by counting the boxes that contained blue dye using ImageJ software. Cardiomyocyte size was determined in 3 randomly chosen sections per sample and the mean size of 30 cardiomyocytes was calculated using AxioVision Rel 4.6 (Carl Zeiss AG, Feldbach, Switzerland) at 40× magnification.

RNA extraction and microarray analysis

Microarray analysis was carried out in hearts of C57BL/6J and 129S1/SvImJ mice ten weeks or twelve weeks following Sham or TAC surgery, respectively, and compared to samples obtained from failing or non-failing human left ventricles. Total RNA was isolated from hearts with TRIzol reagent (Invitrogen Corporation, Carlsbad, CA) and purified with the RNeasy Kit (Qiagen, Hilden, Germany), as described before.[29] RNA integrity was analyzed by capillary electrophoresis using a Fragment Analyzer (Advanced Analytical Technologies, Inc., Ames, IA). RNA samples had RNA quality numbers (RQN) between 7.8

and 9.1 and were further processed with the Affymetrix Clariom D assays as described by the manufacturers. Partek Genomics Suite software was used for analysis (Partek Inc., St. Louis, MO). CEL files were imported including control and interrogating probes, pre-background adjustment was set to adjust for GC content and probe sequence, and Robust Multi-array Average (RMA) background correction was performed. Arrays were normalized using quantile normalization and probe set summarization was done using Median Polish. Probe values were log2 transformed. Differentially expressed genes between groups were identified using a 1-way ANOVA in Partek Genomics Suite software. Contrasts between groups were performed using Fisher's Least Significant Difference as contrast method.

Statistical Analysis and visualization of microarray data

To understand the global effects of heart failure on gene transcription, differential expression analysis of the microarray data was performed using several levels of stringency. The first low-stringency threshold of $P < 0.01$ to visualize the global changes that accompany each model independent of expression magnitude. The second threshold of $P < 0.05$ with $|\text{fold-change}| > 1.5$ was then used to identify the specific genes that (1) are differentially expressed in each model and therefore (2) determine the potential usefulness of each mouse strain to model the human disease. Using this 50% change in gene expression as our threshold permitted an experimental variance of 0.72 to detect a true difference with a sample size $n=5$, power=0.80, and Type 1 error probability $\alpha=0.10$. The highest statistical stringency was reserved for gene set enrichment analysis and pathway enrichment, for which we incorporated the use of Benjamini-Hochberg (B-H) correction to adjust for multiple comparisons. Doing so provided us with the needed confidence to cluster genes into known pathways and identify putative upstream regulators for each comparison. Due to the low number of genes reaching statistical significance for the 129S1/SvImJ TAC vs. Sham comparison, the results of this analysis are reserved as supplemental data.

To appreciate and visualize the global transcriptional changes that occur in the heart failure model for both mouse strains, microarray of mouse TAC vs. Sham were sorted according to a reduced-stringency criterion ($P < 0.05$). To infer relevance of each mouse strain to the human cardiac phenotype, only known and annotated genes with homology to the human genome were used in this comparison. Homologous genes in the human heart failure comparison were then filtered by the list of differentially-expressed mouse genes, and merged onto the mouse dataset. All beta values were then normalized to the respective mouse and human non-failing controls to allow for both cross-species and cross-platform comparison. Heatmap visualization of the Sham-normalized beta values was performed using R (version 3.4.2) package *pheatmap* (version 1.0.8) using differentially expressed genes achieving $P < 0.05$ by Fisher's exact test in the microarray. To the heatmap we applied hierarchical clustering, which enables samples to be grouped using an iterative process that calculates the level of dissimilarity among all objects (i.e. samples). Specifically, we used the Ward's minimum variance algorithm (D2), with dendrogram length defined by Euclidean distance.

Gene Set Enrichment and Pathway Analysis

Unfiltered differential expression data of all 3 comparisons were merged into a single data matrix by annotated gene name, and the compiled dataset was then imported into the Ingenuity Pathway Analysis software (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity). Due to the IPA software's necessity for a gene identifier (such as gene name or RefSeq Gene ID) to perform pathway enrichment analysis, transcripts lacking a known gene were necessarily removed from the dataset. Annotating the dataset reduced the table size from 72,689 to 39,601 transcripts. The transcripts were then annotated within IPA by (1) known genes via RefSeq Gene ID, and (2) genes possessing a known homologous gene in humans. This processing further reduced the numbers to our analysis-ready dataset totaling 10,015 genes for all comparisons. Within IPA, a significance threshold of $P < 0.05$ and $|\text{fold-change}| > 1.5$ was established for each of the 3 comparisons (Table S2). These genes were then used to generate the following enrichment analyses: Canonical Pathways, Upstream Analysis, Diseases and Functions, Regulator Effect, Gene Networks, and Tox Lists (Tables S3–S5 for each data set). For canonical pathway analysis, pathway enrichment was reported as percent of total genes that annotate to a given pathway, along with the Bonferroni-Hochberg (B–H)-adjusted P -value.

Statistical Analysis

Data are presented as means $\pm$ SE. When comparing two groups, significance was determined using a Student's t -test using GraphPad Prism 7 software (GraphPad Software, Inc., La Jolla, CA). When comparing more than two groups, a 2-way analysis of variance (ANOVA) was performed followed by a Bonferroni post-hoc analysis. Significant difference was accepted when $P < 0.05$. Statistical analysis of microarrays was performed as described in "Statistical Analysis and visualization of microarray data".

Results

Cardiac function and remodeling in C57BL/6J and 129S1/SvImJ mice following TAC

Male, 8-week-old C57BL/6J and 129S1/SvImJ mice were subjected to minimally-invasive TAC surgery or a Sham procedure, and mice undergoing TAC received clips with different clip sizes. None of the mice died before the planned date of sacrifice. C57BL/6J mice received clip application with clip sizes of 27G, 30G, or 32G, and were observed for 10 weeks by echocardiography every 2 weeks. While C57BL/6J mice undergoing TAC with a clip diameter of 27G or 30G did not show a significant impairment of cardiac function, C57BL/6J mice that received a 32G clip showed a progressive decline in ejection fraction, starting as early as 4 weeks following TAC (Fig. 1a, Fig. S1, Table S14). The decrease in cardiac function was accompanied by a progressive increase in LV end-systolic volume (Fig. 1b), indicating cardiac dilation, and mean values of lung weight-to-body weight ratios at sacrifice were increased ($P = 0.09$), pointing towards the development of pulmonary edema (Fig. 1e, Fig. S2, Table S6). In contrast, 129S1/SvImJ required a 34G clip to induce an impairment in ejection fraction, which did not occur until 8 weeks following TAC (Fig. 1c, Fig. S3, Table S15). Furthermore, end-systolic volume did not increase in these mice over time (Fig. 1d, Fig. S4). In contrast to C57BL/6J mice, heart weight/body weight ratios (+ 14 %) were not significantly increased, and lung weight/body weight ratios were

completely unchanged in 129S1/SvImJ mice following TAC (Fig. 1f, Table S7). Of note, although mean values for maximal pressure gradients were lower in 129S1/SvImJ mice following 34G clip application compared to C57BL/6J mice following 32G clip application (Fig. S5), the relative increase of pressure gradients following TAC was similar in both strains compared to their respective Sham operated littermates (Fig. 1g). On the molecular level, C57BL/6J mice showed a switch of MHC isoforms towards increased α -MHC expression, which did not occur in 129S1/SvImJ mice (Fig. 2a, b). Expression of ANP was increased in both strains following TAC, but exaggerated in C57BL/6J mice (Fig. 2a, b). As observed for heart weight/body weight ratios, cardiomyocyte size was increased to a greater extent in C57BL/6J mice compared to 129S1/SvImJ mice following TAC (Fig. 2c). Myocardial collagen content was increased to the same extent in both strains (Fig. 2d). Staining for CD31, Ki-67, MAC3, and TUNEL staining showed no differences between Sham and TAC operated C57BL/6J and 129S1/SvImJ mice (Fig. 2e–h), suggesting no differences in capillary density, cell proliferation, macrophage infiltration and apoptosis between mouse strains following TAC. Thus, minimally-invasive TAC using micro clip application was sufficient to induce cardiac dysfunction in both genetic strains, but only C57BL/6J mice developed overt heart failure with less aortic constriction and earlier onset of contractile defects, compatible with increased susceptibility of C57BL/6J mice for heart failure development.

Differential gene expression in C57BL/6J and 129S1/SvImJ mice following TAC

To understand which mechanisms may contribute to the differential susceptibility to pressure overload-induced heart failure in these two strains, we performed a gene expression analysis in hearts of C57BL/6J mice that received a 32G clip and developed overt heart failure, and in hearts of 129S1/SvImJ mice that received a 34G clip and developed cardiac dysfunction, respectively. Affymetrix microarray analysis was performed to quantify differential gene expression, in which 39,601 coding regions were quantified. Of these, 277 genes reached significance in 129S1/SvImJ mice (TAC vs. Sham), with 71 genes reaching the defined threshold ($P < 0.05$ and $|\text{fold-change}| > 1.5$; for detailed gene list see Table S2). In C57BL/6J mice (TAC vs. Sham), however, a more robust transcriptional profile emerged, yielding 2,490 significantly regulated genes and 461 genes reaching the defined threshold ($P < 0.05$ and $|\text{fold-change}| > 1.5$; for detailed gene list see Table S2). When comparing data among strains, 51 genes were regulated in both strains, all of which were upregulated (Fig. 3a; for detailed gene list see Table S8). No genes were downregulated or contra-regulated between strains. When looking at uniquely regulated genes, 417 genes were upregulated and 25 genes were downregulated in C57BL/6J mice, whereas 25 genes were upregulated and no genes were downregulated in 129S1/SvImJ mice (Fig. 3a; for detailed gene list see Table S8).

Gene ontology term enrichment analysis

Because uniquely regulated genes may contribute to the observed differential susceptibility for heart failure development between the two mouse strains, we performed a Gene Ontology (GO) term enrichment analysis for each strain. Per gene enrichment analysis, C57BL/6J mice exhibited a robust differential gene expression signature consistent with adverse cardiac remodeling and ventricular dysfunction. When examining the Toxicity Network, the enrichment analysis revealed "cardiac hypertrophy" as the pathway that

showed the most robust association (31 genes) with the differential gene expression pattern of C57BL/6J mice, while only 6 genes matched this pathway in 129S1/SvImJ mice (Fig. 3b and Table S9). Of these genes, periostin (*Postn*) was the most highly regulated gene in both strains, exhibiting a 16.7-fold upregulation in C57BL/6J mice, whereas expression was increased by 3.7-fold in 129S1/SvImJ mice (Fig. 3c). In addition, a number of genes associated with ventricular remodeling and oxidative stress were identified as uniquely regulated in C57BL/6J mice, including NADPH oxidase 4 (*Nox4*), connective tissue growth factor (*Ctgf*), fibronectin 1 (*Fnl*), and matrix metalloproteinase 2 (*Mmp2*) (Fig. 3c). To identify the differentially expressed genes that may contribute to differential susceptibility for heart failure, and to also include differentially regulated genes that may enrich in other pathways of cardiac remodeling and failure, we searched PubMed for evidence that the 51 genes co-regulated in both mouse strains have known association with heart failure development. Only published studies providing a mechanistic design have been included in the analysis. Based on these criteria, 4 of the 51 genes commonly regulated in both strains are known to contribute to the development of heart failure: *Postn*, follistatin-like 1 (*Fstll*), signal transducer and activator of transcription 3 (*Stat3*), and transglutaminase 2 (*Tgm2*) (Table 1). Of note, 2 of these genes (*Postn* and *Stat3*) showed clearly higher expression levels in C57BL/6J mice compared to 129S1/SvImJ mice, but only increased expression of *Postn* can be predicted to exert maladaptive effects in heart failure (Table 1 and Table S10 for references). In contrast to commonly regulated genes between strains, 22 of the uniquely regulated genes in C57BL/6J mice could be linked to the development of heart failure (Table 2). Based on the current published literature, it is predicted that 14 of these 22 genes may serve a maladaptive function in the development of heart failure, whereas 8 of the 22 genes may serve an adaptive function (Table 2 and Table S10 for references). 4 of the 14 genes (*Ctgf*, *Nox4*, *Fnl*, G protein-coupled receptor kinase 5 (*Grk5*)) predicted to exert maladaptive effects were also identified for C57BL/6J mice to be associated with cardiac hypertrophy (Fig. 3c). In contrast to C57BL/6J mice, only 1 gene, prolyl 4-hydroxylase subunit alpha 1 (*P4ha1*), was uniquely regulated in 129S1/SvImJ mice, and our PubMed search would predict maladaptive effects (Table 2). The uniquely increased expression of nuclear receptor subfamily 4 group A member 1 (*Nr4a1*) and of general transcription factor 2B (*Gtf2b*) in 129S1/SvImJ can be linked to cardiac hypertrophy development, but sufficient evidence for a clear attribution whether these changes may be maladaptive or protective is lacking. Thus, the two interrogated mouse strains show distinct differences in the gene expression profile, with C57BL/6J mice exhibiting a more robust association with a gene expression signature observed in and predictive for heart failure.

Differential gene expression patterns analysis between human and murine heart failure

To determine whether the differentially expressed genes in the murine models corresponded to those found in the human failing heart, microarray analysis of human failing and non-failing hearts was completed. Left ventricular samples of 5 patients with systolic heart failure due to dilated cardiomyopathy were analyzed for differential gene expression using the same microarray analysis as performed in mice, using 5 samples of non-failing (NF) hearts as controls. Patients with heart failure had a mean ejection fraction of 19%, had medical treatment according to current heart failure guidelines, and medical records did not contain any evidence of coronary artery disease, hypertension, or diabetes mellitus.

Hierarchical clustering and heat map analysis were then completed to identify global transcriptional patterns consistency between each mouse model and the human heart samples by phenotype (i.e. HF or NF). The resulting heatmaps demonstrate that the transcriptional changes in C57BL/6J mice tightly group with human hearts according to cardiovascular phenotype (i.e. HF and TAC; Fig. 4, Left; and Table S11). In contrast, the transcriptional changes noted in the 129S1/SvImJ mouse model failed to generate a distinction between failing and non-failing hearts (Fig. 4, Right; and Table S11).

Identification of differentially expressed genes related to heart failure development

To specifically identify how many and which genes were driving the similarities and differences, all comparisons (human HF vs. NF, C57BL/6J TAC vs. Sham, and 129S1/SvImJ TAC vs. Sham) were overlain to identify lists of genes that are common and distinct using $P < 0.05$ and $|\text{Fold-Change}| > 1.5$ (Fig. 5a and Table S12), as shown in the Venn diagram, which was generated by VennPlex [5]. Of the commonly regulated genes, 12 were upregulated in all comparisons, including POSTN and FSTL1, which we already identified in the comparison of the mouse strains (Fig. 5b and Table S12). Of the remaining 10 genes, several are known to affect cardiac physiology: cysteine-rich angiogenic inducer 61 (CYR61), which may be essential for cardiac development; [43] four-and-a-half LIM domains 1 (FHL1), lack of which may blunt the hypertrophic response following TAC and mutations of which have been implicated in the development of human hypertrophic cardiomyopathy [15, 58]; lumican, which exerts pro-fibrotic effects and may increase the mRNA content of collagen type I alpha 2, lysyl oxidase and transforming growth factor- β 1 mRNA [13]; transferrin receptor (TFRC), cardiomyocyte-specific deletion of which results in early postnatal lethality [72]. A total of 54 genes were differentially co-expressed in human and C57BL/6J failing hearts (Fig. 5a). Based on our PubMed literature search, 5 upregulated genes have been previously linked to heart failure development and may all serve a maladaptive function: connective tissue growth factor (CTGF), fibronectin 1 (FN1), lysyl oxidase (LOX), NADPH oxidase 4 (NOX4) and transforming growth factor β 2 (TGF β 2). Only 4 genes were differentially co-expressed in human and 129S1/SvImJ failing hearts, but none of these were previously linked to heart failure development.

Identification of differentially regulated pathways related to heart failure development

Following identification of differentially regulated genes related to heart failure development, we next aimed to determine whether the transcriptional changes of the two mouse strains in response to TAC corresponded to certain molecular pathways with documented relevance to human heart failure. Due to low overall gene numbers, the 129S1/SvImJ mouse model was unable to populate the known pathways to achieve sufficient statistical significance by Bonferroni-adjusted P -value. Therefore, the pathway enrichment analysis became a focused assessment of C57BL/6J mice (TAC vs. Sham) as a descriptive model for human heart failure. Accordingly, lists of statistically-significant putative upstream regulators for both Human and C57BL/6J were merged and ranked according to absolute Z -score magnitude (Fig. 5c). From this analysis, angiotensin was exposed as the top shared candidate regulator governing the transcriptional changes between human HF and C57BL/6J TAC mice ($P < 0.05$, $|\text{fold-change}| > 1.5$). As shown in the gene map visualizing the signaling components of angiotensin signaling in Fig. 5d, $>75\%$ (18/23) genes showed a

common upregulation (POSTN, NPR3, COL4A1, NOX4, NPPB, TNFRSF12A, TGFB2, MMP2, FN1, BCL2, CTGF, ITGA5, NPPA, IGFBP3, JUN, SERPINE1, MAP1B, and TFRC) in human failing and C57BL/6J failing hearts, with only 5 genes inversely regulated (STAT3, ETS1, GNAO1, TBX20, and BCL2L1). Interestingly, 4 of the 5 genes differentially co-expressed in human and C57BL/6J failing hearts (NOX4, TGFB2, FN1, CTGF) linked to the development of HF were also identified as components of angiotensin signaling, suggesting increased myocardial angiotensin signaling as a central mediator of maladaptive remodeling in human heart failure. Furthermore, these 4 genes were also uniquely regulated in C57BL/6J mice versus 129S1/SvImJ mice, suggesting increased angiotensin signaling as a major contributor to increased susceptibility for HF development in C57BL/6J mice. The second most significant upstream regulator of differential gene expression was TGF β 1 signaling.

To further define the similarities between C57BL/6J failing hearts and human failing hearts, additional enrichment of genes was performed and related to known canonical pathways. Of note, well-known pathways found common to these data sets highlights a list of known contributors to the pathogenesis of human heart failure among the top 10 pathways: IGF-1, PI3K/AKT, Insulin Receptor, and Cardiac Hypertrophy signaling (Fig. 6a; for complete pathway list see Table S13). Of these, IGF-1 signaling was identified as the most robustly represented pathway among the various comparisons. Of the 21 enriched genes in human HF and the 11 enriched genes in murine HF, 10 genes showed a common upregulation in murine and human failing hearts, whereas only one gene was contra-regulated (Fig. 6b).

Discussion

In humans, genetic heterogeneity and genetic factors influence the risk of developing heart failure. To further understand the importance and pathways by which alterations in gene expression may contribute to heart failure susceptibility, we took advantage of the differential susceptibility of C57BL/6J and 129S1/SvImJ mice to develop cardiac dysfunction and overt heart failure following chronic exposure to pressure overload induced by TAC and analyzed the myocardial gene expression profiles [4]. As expected, cardiac dysfunction occurred earlier and remodeling was more pronounced in C57BL/6J mice following TAC, even though the degree of aortic constriction was less compared to 129S1/SvImJ mice that underwent TAC. While our survey using different clip diameters for TAC should provide a reliable guide for researchers what microclip diameter is sufficient to induce cardiac dysfunction and failure in these two commonly used mouse strains using microclip-based minimally-invasive TAC [52], even more important is to stress the dependency of TAC-induced heart failure phenotype on genetic strain and the potential consequences for data interpretation. Embryonic stem (ES) cell lines used for genetic engineering of mice have been developed from a number of inbred mouse strains, which for technical reasons were mainly derived from mice on a 129Sv, C57BL/6J or BALB/c genetic background [10, 14, 40]. As a consequence, many of the genetically modified mouse lines available today are maintained on these genetic strains, which are well known to differ in their physiologic phenotype and susceptibility for disease development, including cardiovascular disease [4, 45, 54, 57]. Thus, our study reinforces two important issues to be considered in animal model-based research: 1) Genetic background has strong effects on

cardiac phenotype, reinforcing the importance of a complete backcross of ES-derived genetically manipulated mice into an inbred strain to avoid genetic drifting which may influence the outcome of experiments; 2) The extent to which the gain or loss of specific gene function affects cardiac function and/or development of heart failure in response to TAC strongly depends on the genetic background strain. Thus, caution is warranted when drawing general conclusions from genetically engineered mice not to overestimate, underestimate, or misinterpret the effects of gene manipulation. In fact, experiments in outbred strains may resemble human genetic heterogeneity more closely and significant effects may be more likely reproducible in humans compared to data generated in inbred strains with a rather selective disease susceptibility. Based on our hierarchical clustering and heatmap analysis that allows identification of human and murine heart failure by differential gene expression patterns, we conclude that TAC-induced changes in gene expression and cardiac function in C57BL/6J mice may resemble the human heart failure phenotype more closely than 129S1/SvImJ mice. Our findings also suggest that screens of additional strains of mice and possibly outbred mice are warranted depending on the specific human pathway the investigator is trying to model.

To further understand which specific genes may drive increased heart failure susceptibility, we performed a GO term enrichment analysis and a manual PubMed literature search to predict the function of specific gene expression changes. Five genes were differentially co-expressed in C57BL/6J mice and human heart failure: NOX4, LOX, CTGF, FN1, and TGF β 2. Overexpression of each of these genes can be predicted to contribute to heart failure development. Lack of NOX4 in cardiomyocytes results in attenuated cardiac hypertrophy, less interstitial fibrosis and apoptosis, less oxidative stress and less impairment of cardiac function in response to chronic pressure overload, whereas overexpression of NOX4 exacerbates cardiac dysfunction, fibrosis, and apoptosis [34]. Pharmacological inhibition of LOX attenuates fibrosis and cardiac dysfunction in response to chronic volume overload [11]. Antagonizing antibody treatment for CTGF attenuated cardiac dysfunction, dilation and hypertrophy following TAC [62]. Loss of FN1 in mice attenuates cardiac hypertrophy and contractile dysfunction in response to chronic pressure overload, whereas FN1 induces hypertrophy in neonatal rat cardiomyocytes [32]. Knockdown of TGF β 2 receptor in cardiomyocytes attenuates TAC-induced fibrosis, hypertrophy and contractile dysfunction [31]. Interestingly, NOX4, CTGF and FN1 were also identified as uniquely regulated genes in C57BL/6J mice compared to 129S1/SvImJ mice. Thus, the above-mentioned genes are likely candidates that may promote heart failure development in humans and may contribute to increased heart failure susceptibility in C57BL/6J mice compared to 129S1/SvImJ mice.

As a result of our combined-enrichment pathway analysis in murine and human heart failure, we identified angiotensin signaling as the most significant upstream regulator of differential gene expression. Angiotensin II is the major effector molecule of the renin-angiotensin-aldosterone system (RAAS), which is strongly activated both systemically and locally in heart failure. While increased angiotensin II signaling represents a plausible underlying mechanism of differential susceptibility for heart failure between the mouse strains, it remains to be discovered why RAAS-regulated cardiac gene expression is different between strains. One hypothesis may be related to anatomical differences between strains. Activation of the RAAS is the result of reduced renal perfusion, and ejection fraction was relatively

lower and dropped earlier in C57BL/6J mice following TAC compared to 129S1/SvImJ mice. Thus, the observed changes in myocardial gene expression may simply reflect the duration and intensity of RAAS activation. In fact, several studies demonstrated differences in aortic arch geometry between C57BL/6J and 129S1/SvImJ mice, including a higher diameter of the ascending aorta in C57BL/6J mice [65, 75]. If using the same microclip size, C57BL/6J mice would then be expected to experience a stronger relative reduction of aortic diameter by TAC than 129S1/SvImJ mice. As a consequence, these mice may develop a relatively higher degree of pressure overload, which could explain the accelerated development of heart failure and stronger activation of the RAAS system compared to 129S1/SvImJ mice. While absolute values of peak pressure gradients across the constriction site in the aortic arch were higher in C57BL/6J than in 129S1/SvImJ mice (Fig. S5), the relative increase in pressure gradients was similar between the two mouse strains following TAC (Fig. 1g), rather suggesting similar changes in renal perfusion and activation of RAAS. Of note, when comparing to suture-mediated TAC in mice with comparable development of cardiac dilation and dysfunction (i.e. 27G suture-mediated TAC in C57BL/6J mice [4, 44], absolute values of pressure gradients were lower in our study, potentially implying a relatively lower degree of pressure overload despite using a tighter (32G) diameter in C57BL/6J mice. This difference may however be related to differences in anatomical shape of the constricted site (clip-mediated TAC with oval area of constriction versus suture-mediated TAC with round-shaped area of constriction) which may result in different hemodynamics and different results for Doppler measurements of pressure gradients.

A second hypothesis for different RAAS-regulated cardiac gene expression between strains is one of genetic variance. This could contribute to the anatomical differences between strains. Several chromosomal regions have been identified in mice that determine geometry of the aortic arch and may explain geometric differences between C57BL/6J and 129S1/SvImJ mice, supporting the concept that genetic factors also drive aortic geometry [66]. Or the genetic variance could contribute to altered signaling. Specifically, the variants affecting the levels and activity of proteins and enzymes that comprise the RAAS may contribute to heart failure susceptibility between strains. For example, ACE and AT1R gene loci may carry alleles influencing blood pressure variation in people with essential hypertension [20]. The DD polymorphism in intron 16 of the ACE gene in humans might be a risk factor for cardiac hypertrophy, myocardial infarction, and ischemic and idiopathic dilated cardiomyopathy [6, 21, 50]. Also, gene variants of renin and angiotensinogen have been linked to hypertension development in rats and humans [23, 49, 69]. Mostly likely, however, the differences are a combination of both the variance in structure and genetic plasticity similar to the heterogeneity of disease progression seen in different individuals.

The POSTN gene showed the strongest upregulation of all genes in both mouse strains, achieving a 16.7-fold increase in C57BL/6J mice. The gene product periostin, is an extracellular matrix protein; the attenuation of which in cardiomyocytes reduces fibrosis and hypertrophy in response to long-term pressure overload, whereas overexpression of periostin resulted in age-dependent hypertrophy. These data strongly suggest that overexpression of periostin significantly contributes to cardiac remodeling following TAC [1, 47]. POSTN expression was also upregulated in LV samples of human failing hearts in our study (by 5.5-fold) and in other studies, and POSTN expression is negatively correlated with LV ejection

fraction and positively correlated with end-diastolic diameter following myocardial infarction, suggesting that increased POSTN expression may contribute to cardiac contractile impairment in humans [8, 74]. In addition, the higher extent of POSTN expression in C57BL/6J versus 129S1/SvImJ mice could also partially contribute to the accelerated transition to HF in C57BL/6J mice. Increased expression of POSTN may be driven by increased angiotensin signaling (Fig. 5d) activating the Ras/p38 MAPK/CREB pathway and/or ERK/TGF β 1/SMAD signaling [38]. Besides POSTN, TGM2 was co-expressed in C57BL/6J and 129S1/SvImJ mice and may contribute to cardiac dysfunction and remodeling in both strains since cardiomyocyte-selective overexpression of TGM2 leads to cardiac hypertrophy, increased fibrosis and contractile dysfunction [60]. In contrast, co-expression of FSTL1 and STAT3 in the two mouse strains may exert initial protective functions in cardiac hypertrophy and failure. Overexpression of FSTL1 or treatment with FSTL1 attenuates cardiac hypertrophy and dysfunction in response to TAC and chronic aldosterone infusion, and cardiomyocyte-specific deletion of STAT3 results in fibrosis and heart failure with increasing age or in response to chronic isoproterenol infusion [22, 59, 61, 63].

Two of the uniquely upregulated genes in 129S1/SvImJ versus C57BL/6J mice following TAC were *Nr4a1* and *Gtf2b*. *Nr4a1* encodes for an orphan nuclear receptor (also termed Nur77) which regulates the cellular stress response, metabolism, inflammation, apoptosis and cell proliferation. In the heart, Nur77 may exhibit both protective and maladaptive effects depending on the type of cardiac insult. Nur77 expression is induced by angiotensin II signaling, is increased in heart failure, and deletion of Nur77 in mice may attenuate cardiac hypertrophy, maintain fractional shortening, and prevent fibrosis and apoptosis in response to angiotensin II-induced pressure overload [26, 70]. Similarly, remodeling was attenuated in Nur77^{-/-} mice in response to TAC-induced pressure overload [42]. In contrast and consistent with protective effects, lack of Nur77 in hematopoietic cells aggravates the inflammatory response and impairs healing during myocardial infarction [19]. Furthermore, overexpression of Nur77 inhibited cardiac hypertrophy and prevented cardiac dysfunction induced by isoproterenol treatment, whereas deletion of Nur77 resulted in increased hypertrophy and fibrosis in response to isoproterenol treatment [42, 73]. Whether increased Nur77 expression is protective or maladaptive in 129S1/SvImJ mice following TAC remains to be elucidated.

GTF2B is a general transcription factor that is required for the recruitment of RNA polymerase II (Pol II) to promoter start sites and thus for the initiation of transcription of all promoters. Recently, it was shown that GTF2B was constitutively bound to Pol II at paused promoters regulating the expression of housekeeping genes in the heart, whereas de novo recruitment of GTF2B and Pol II was required for specialized genes that are induced during cardiac hypertrophy [55]. Inhibition of induction of the latter set of genes using locked nucleic acid (LNA)-modified antisense GTF2B oligonucleotide treatment resulted in attenuation of cardiac hypertrophy [56]. Thus, increased expression of GTF2B in 129S1/SvImJ mice following TAC may contribute to different expression profiles between the two mouse strains in response to TAC.

As a result of our GO term enrichment analysis, IGF-1 signaling was identified as the top regulated canonical pathway common between the C57BL/6J model and human heart failure. In this pathway, all of the 11 genes differentially regulated by TAC in C57BL/6J mice were also changing in human heart failure, and 10 were upregulated both in C57BL/6J mice and human failing hearts. Only STAT3 was inversely regulated between groups. IGF-1 is a key regulatory protein of cellular energy metabolism with largely overlapping functions with insulin signaling, and IGF-1 is required for physiological hypertrophy in response to chronic exercise [27]. Conversely, recent studies also implicated IGF-1 deficiency in increased longevity in aged mice, potentially due to prevention of aging-associated cardiac hypertrophy, attenuated myocardial inflammation and decreased fibrosis due to inhibition of AKT signaling [46]. In heart failure, IGF-1 serves as a key prognostic neurohormonal marker, and in most studies serum levels of IGF-1 seem to be reduced [3]. Thus, the overall pattern of increased expression of IGF-1 signaling components may imply a compensatory response to reduced IGF-1 serum levels. In this sense, impaired IGF-1 signaling may contribute to cardiac hypertrophy, contractile dysfunction, increased apoptosis, decreased protein synthesis and increased protein degradation, and impaired glucose utilization, among other mechanisms [67]. Of interest, overexpression of IGF-1 in cardiomyocytes downregulates local angiotensin 2 levels in the heart and attenuates cardiac dysfunction, dilation, hypertrophy and cell death in a murine heart failure model [35, 71]. Thus, it is tempting to speculate that impaired IGF-1 signaling may contribute to exacerbation of cardiac dysfunction in response to pressure overload, possibly by disinhibition of the local RAAS in the heart. It may be speculated that increasing IGF-1 signaling may represent a promising therapeutic approach to be explored in the setting of heart failure.

One of the limitations of our study is that we used samples from humans that suffered from DCM but had no adverse effects from chronic pressure overload (no hypertension, no significant valvular stenosis), thus complicating a direct extrapolation of our findings from C57BL/6J mice to the human condition. Nevertheless, we identified several commonly regulated genes predicted to exhibit maladaptive effects, and due to the different etiology of HF in mice and humans, these genes may thus contribute to HF development even independent of HF etiology and thus represent potential therapeutic targets of higher priority and for a larger cohort of HF patients. Another limitation may be that 129S1/SvImJ mice did not develop overt HF but only compensated hypertrophy, maybe since the time of observation following TAC was too short. Thus, we cannot exclude that the more pronounced changes in gene expression and thus the more robust association with a gene expression signature observed in and predictive for heart failure may be simply related to the severity of cardiac dysfunction and heart failure in our mouse models.

In conclusion, we provide evidence that TAC-induced heart failure in C57BL/6J mice more accurately replicates the gene expression pattern of human dilated cardiomyopathy compared to 129S1/SvImJ mice and may thus represent the model of choice for studies focusing on gene expression therapy to combat heart disease. Our unbiased as well as targeted gene expression and pathway analyses identified a number of genes which may be causally linked to the development of heart failure in human DCM and to increased HF susceptibility between mouse strains, including POSTN, NOX4, TGF β 2, FN1 and CTGF. Angiotensin signaling has been identified as a major upstream regulator that seems to

coordinately increase the expression of several genes previously linked to heart failure development, including the aforementioned, emphasizing the importance of early RAAS inhibition in the development of heart failure to prevent adverse effects on the heart.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Dietmar Pfeifer at the University of Freiburg for technical assistance in performing microarray analyses.

Sources of funding

This study was supported by a research grant of the Deutsche Forschungsgemeinschaft to H.B. (Bu2126/3-1), and by a National Institutes of Health (NIH) grant to A.R.W. (HL133011). Training support was provided by NIH T32 to M.E.P. (T32HD071866).

References

- Ameling S, Bhardwaj G, Hammer E, Beug D, Steil L, Reinke Y, Weitmann K, Grube M, Trimper C, Klingel K, Kandolf R, Hoffmann W, Nauck M, Dorr M, Empen K, Felix SB, Volker U. Changes of myocardial gene expression and protein composition in patients with dilated cardiomyopathy after immunoadsorption with subsequent immunoglobulin substitution. *Basic Res Cardiol*. 2016; 111:53.doi: 10.1007/s00395-016-0569-y [PubMed: 27412778]
- Arany Z, Novikov M, Chin S, Ma Y, Rosenzweig A, Spiegelman BM. Transverse aortic constriction leads to accelerated heart failure in mice lacking PPAR-gamma coactivator 1alpha. *Proc Natl Acad Sci U S A*. 2006; 103:10086–10091. DOI: 10.1073/pnas.0603615103 [PubMed: 16775082]
- Arcopinto M, Bobbio E, Bossone E, Perrone-Filardi P, Napoli R, Sacca L, Cittadini A. The GH/IGF-1 axis in chronic heart failure. *Endocr Metab Immune Disord Drug Targets*. 2013; 13:76–91. DOI: 10.2174/1871530311313010010 [PubMed: 23369140]
- Barrick CJ, Rojas M, Schoonhoven R, Smyth SS, Threadgill DW. Cardiac response to pressure overload in 129S1/SvImJ and C57BL/6J mice: temporal- and background-dependent development of concentric left ventricular hypertrophy. *Am J Physiol Heart Circ Physiol*. 2007; 292:H2119–2130. DOI: 10.1152/ajpheart.00816.2006 [PubMed: 17172276]
- Cai H, Chen H, Yi T, Daimon CM, Boyle JP, Peers C, Maudsley S, Martin B. VennPlex--a novel Venn diagram program for comparing and visualizing datasets with differentially regulated datapoints. *PLoS One*. 2013; 8:e53388.doi: 10.1371/journal.pone.0053388 [PubMed: 23308210]
- Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Ricard S, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature*. 1992; 359:641–644. DOI: 10.1038/359641a0 [PubMed: 1328889]
- Carnethon MR, Pu J, Howard G, Albert MA, Anderson CAM, Bertoni AG, Mujahid MS, Palaniappan L, Taylor HA Jr, Willis M, Yancy CW, American Heart Association Council on E, Prevention, Council on Cardiovascular Disease in the Y, Council on C, Stroke N, Council on Clinical C, Council on Functional G, Translational B, Stroke C. Cardiovascular Health in African Americans: A Scientific Statement From the American Heart Association. *Circulation*. 2017; doi: 10.1161/CIR.0000000000000534
- Cheng CW, Wang CH, Lee JF, Kuo LT, Cherng WJ. Levels of blood periostin decrease after acute myocardial infarction and are negatively associated with ventricular function after 3 months. *J Invest Med*. 2012; 60:523–528. DOI: 10.2310/JIM.0b013e3182408549
- Dassanayaka S, Brainard RE, Watson LJ, Long BW, Brittian KR, DeMartino AM, Aird AL, Gumpert AM, Audam TN, Kilfoil PJ, Muthusamy S, Hamid T, Prabhu SD, Jones SP. Cardiomyocyte Ogt limits ventricular dysfunction in mice following pressure overload without

affecting hypertrophy. *Basic Res Cardiol*. 2017; 112:23.doi: 10.1007/s00395-017-0612-7 [PubMed: 28299467]

10. Downing GJ, Battey JF. Technical assessment of the first 20 years of research using mouse embryonic stem cell lines. *Stem Cells*. 2004; 22:1168–1180. DOI: 10.1634/stemcells.2004-0101 [PubMed: 15579637]
11. El Hajj EC, El Hajj MC, Ninh VK, Gardner JD. Cardioprotective effects of lysyl oxidase inhibition against volume overload-induced extracellular matrix remodeling. *Exp Biol Med* (Maywood). 2016; 241:539–549. DOI: 10.1177/1535370215616511 [PubMed: 26582054]
12. Eleftheriadou O, Boguslavskyi A, Longman MR, Cowan J, Francois A, Heads RJ, Wadzinski BE, Ryan A, Shattock MJ, Snabaitis AK. Expression and regulation of type 2A protein phosphatases and alpha4 signalling in cardiac health and hypertrophy. *Basic Res Cardiol*. 2017; 112:37.doi: 10.1007/s00395-017-0625-2 [PubMed: 28526910]
13. Engebretsen KVT, Lunde IG, Strand ME, Waehre A, Sjaastad I, Marstein HS, Skrbic B, Dahl CP, Askevold ET, Christensen G, Bjornstad JL, Tonnessen T. Lumican is increased in experimental and clinical heart failure, and its production by cardiac fibroblasts is induced by mechanical and proinflammatory stimuli. *Febs J*. 2013; 280:2382–2398. DOI: 10.1111/febs.12235 [PubMed: 23480731]
14. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature*. 1981; 292:154–156. DOI: 10.1038/292154a0 [PubMed: 7242681]
15. Friedrich FW, Wilding BR, Reischmann S, Crocini C, Lang P, Charron P, Muller OJ, McGrath MJ, Vollert I, Hansen A, Linke WA, Hengstenberg C, Bonne G, Morner S, Wichter T, Madeira H, Arbustini E, Eschenhagen T, Mitchell CA, Isnard R, Carrier L. Evidence for FHL1 as a novel disease gene for isolated hypertrophic cardiomyopathy. *Hum Mol Genet*. 2012; 21:3237–3254. DOI: 10.1093/hmg/dds157 [PubMed: 22523091]
16. Ganau A, Devereux RB, Roman MJ, de Simone G, Pickering TG, Saba PS, Vargiu P, Simongini I, Laragh JH. Patterns of left ventricular hypertrophy and geometric remodeling in essential hypertension. *J Am Coll Cardiol*. 1992; 19:1550–1558. DOI: 10.1016/0735-1097(92)90617-V [PubMed: 1534335]
17. Garcia-Menendez L, Karamanlidis G, Kolwicz S, Tian R. Substrain specific response to cardiac pressure overload in C57BL/6 mice. *Am J Physiol Heart Circ Physiol*. 2013; 305:H397–402. DOI: 10.1152/ajpheart.00088.2013 [PubMed: 23709599]
18. Haider AW, Larson MG, Benjamin EJ, Levy D. Increased left ventricular mass and hypertrophy are associated with increased risk for sudden death. *J Am Coll Cardiol*. 1998; 32:1454–1459. DOI: 10.1016/S0735-1097(98)00407-0 [PubMed: 9809962]
19. Hilgendorf I, Gerhardt LM, Tan TC, Winter C, Holderried TA, Chousterman BG, Iwamoto Y, Liao R, Zirik A, Scherer-Crosbie M, Hedrick CC, Libby P, Nahrendorf M, Weissleder R, Swirski FK. Ly-6C high monocytes depend on Nr4a1 to balance both inflammatory and reparative phases in the infarcted myocardium. *Circ Res*. 2014; 114:1611–1622. DOI: 10.1161/CIRCRESAHA.114.303204 [PubMed: 24625784]
20. Hingorani AD, Jia H, Stevens PA, Hopper R, Dickerson JE, Brown MJ. Renin-angiotensin system gene polymorphisms influence blood pressure and the response to angiotensin converting enzyme inhibition. *J Hypertens*. 1995; 13:1602–1609. [PubMed: 8903618]
21. Iwai N, Ohmichi N, Nakamura Y, Kinoshita M. DD genotype of the angiotensin-converting enzyme gene is a risk factor for left ventricular hypertrophy. *Circulation*. 1994; 90:2622–2628. [PubMed: 7994801]
22. Jacoby JJ, Kalinowski A, Liu MG, Zhang SS, Gao Q, Chai GX, Ji L, Iwamoto Y, Li E, Schneider M, Russell KS, Fu XY. Cardiomyocyte-restricted knockout of STAT3 results in higher sensitivity to inflammation, cardiac fibrosis, and heart failure with advanced age. *Proc Natl Acad Sci U S A*. 2003; 100:12929–12934. DOI: 10.1073/pnas.2134694100 [PubMed: 14566054]
23. Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, Hunt SC, Hopkins PN, Williams RR, Lalouel JM, et al. Molecular basis of human hypertension: role of angiotensinogen. *Cell*. 1992; 71:169–180. DOI: 10.1016/0092-8674(92)90275-H [PubMed: 1394429]
24. Kannel WB. Incidence and epidemiology of heart failure. *Heart Fail Rev*. 2000; 5:167–173. DOI: 10.1023/A:1009884820941 [PubMed: 16228142]

25. Kawahara Y, Tanonaka K, Daicho T, Nawa M, Oikawa R, Nasa Y, Takeo S. Preferable anesthetic conditions for echocardiographic determination of murine cardiac function. *J Pharmacol Sci.* 2005; 99:95–104. DOI: 10.1254/jphs.FP0050343 [PubMed: 16177543]
26. Kelly SN, McKenna TJ, Young LS. Modulation of steroidogenic enzymes by orphan nuclear transcriptional regulation may control diverse production of cortisol and androgens in the human adrenal. *J Endocrinol.* 2004; 181:355–365. [PubMed: 15128284]
27. Kim J, Wende AR, Sena S, Theobald HA, Soto J, Sloan C, Wayment BE, Litwin SE, Holzenberger M, LeRoith D, Abel ED. Insulin-Like Growth Factor I Receptor Signaling Is Required for Exercise-Induced Cardiac Hypertrophy. *Mol Endocrinol.* 2008; 22:2531–2543. DOI: 10.1210/me.2008-0265 [PubMed: 18801929]
28. Kizer JR, Arnett DK, Bella JN, Paranicas M, Rao DC, Province MA, Oberman A, Kitzman DW, Hopkins PN, Liu JE, Devereux RB. Differences in left ventricular structure between black and white hypertensive adults - The hypertension genetic epidemiology network study. *Hypertension.* 2004; 43:1182–1188. DOI: 10.1161/01.HYP.0000128738.94190.9f [PubMed: 15123573]
29. Koentges C, König A, Pfeil K, Holscher ME, Schnick T, Wende AR, Schrepper A, Cimolai MC, Kersting S, Hoffmann MM, Asal J, Osterholt M, Odening KE, Doenst T, Hein L, Abel ED, Bode C, Bugger H. Myocardial mitochondrial dysfunction in mice lacking adiponectin receptor 1. *Basic Res Cardiol.* 2015; 110:37.doi: 10.1007/s00395-015-0495-4 [PubMed: 25982881]
30. Koentges C, Pfeil K, Schnick T, Wiese S, Dahlbock R, Cimolai MC, Meyer-Steenbuck M, Cenkerova K, Hoffmann MM, Jaeger C, Odening KE, Kammerer B, Hein L, Bode C, Bugger H. SIRT3 deficiency impairs mitochondrial and contractile function in the heart. *Basic Res Cardiol.* 2015; 110:36.doi: 10.1007/s00395-015-0493-6 [PubMed: 25962702]
31. Koitabashi N, Danner T, Zaiman AL, Pinto YM, Rowell J, Mankowski J, Zhang D, Nakamura T, Takimoto E, Kass DA. Pivotal role of cardiomyocyte TGF-beta signaling in the murine pathological response to sustained pressure overload. *J Clin Invest.* 2011; 121:2301–2312. DOI: 10.1172/JCI44824 [PubMed: 21537080]
32. Konstantin MH, Volkers M, Collins B, Quijada P, Quintana M, De La Torre A, Ormachea L, Din S, Gude N, Toko H, Sussman MA. Fibronectin contributes to pathological cardiac hypertrophy but not physiological growth. *Basic Res Cardiol.* 2013; 108:375. doi:10.1007/s00395-00013-00375-00398. Epub 02013 Aug 00394. doi: 10.1007/s00395-013-0375-8 [PubMed: 23912225]
33. Kreusser MM, Lehmann LH, Wolf N, Keranov S, Jungmann A, Grone HJ, Müller OJ, Katus HA, Backs J. Inducible cardiomyocyte-specific deletion of CaM kinase II protects from pressure overload-induced heart failure. *Basic Res Cardiol.* 2016; 111:65.doi: 10.1007/s00395-016-0581-2 [PubMed: 27683174]
34. Kuroda J, Ago T, Matsushima S, Zhai PY, Schneider MD, Sadoshima J. NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart. *P Natl Acad Sci USA.* 2010; 107:15565–15570. DOI: 10.1073/pnas.1002178107
35. Leri A, Liu Y, Wang X, Kajstura J, Malhotra A, Meggs LG, Anversa P. Overexpression of insulin-like growth factor-1 attenuates the myocyte renin-angiotensin system in transgenic mice. *Circ Res.* 1999; 84:752–762. DOI: 10.1161/01.RES.84.7.752 [PubMed: 10205143]
36. Levy D, Larson MG, Vasan RS, Kannel WB, Ho KK. The progression from hypertension to congestive heart failure. *JAMA.* 1996; 275:1557–1562. DOI: 10.1001/jama.1996.03530440037034 [PubMed: 8622246]
37. Li CY, Zhou Q, Yang LC, Chen YH, Hou JW, Guo K, Wang YP, Li YG. Dual-specificity phosphatase 14 protects the heart from aortic banding-induced cardiac hypertrophy and dysfunction through inactivation of TAK1-P38MAPK/-JNK1/2 signaling pathway. *Basic Res Cardiol.* 2016; 111:19.doi: 10.1007/s00395-016-0536-7 [PubMed: 26891723]
38. Li L, Fan D, Wang C, Wang JY, Cui XB, Wu D, Zhou Y, Wu LL. Angiotensin II increases periostin expression via Ras/p38 MAPK/CREB and ERK1/2/TGF-beta1 pathways in cardiac fibroblasts. *Cardiovasc Res.* 2011; 91:80–89. DOI: 10.1093/cvr/cvr067 [PubMed: 21367774]
39. Li Y, Tang XH, Li XH, Dai HJ, Miao RJ, Cai JJ, Huang ZJ, Chen AF, Xing XW, Lu Y, Yuan H. Regulator of G protein signalling 14 attenuates cardiac remodelling through the MEK-ERK1/2 signalling pathway. *Basic Res Cardiol.* 2016; 111:47.doi: 10.1007/s00395-016-0566-1 [PubMed: 27298141]

40. Limaye A, Hall B, Kulkarni AB. Manipulation of mouse embryonic stem cells for knockout mouse production. *Curr Protoc Cell Biol.* 2009; :11–24. Chapter 19:Unit 19 13 19 13. DOI: 10.1002/0471143030.cb1913s44 [PubMed: 19283729]
41. Mayet J, Shahi M, Foale RA, Poulter NR, Sever PS, Mc GTSA. Racial differences in cardiac structure and function in essential hypertension. *BMJ.* 1994; 308:1011–1014. [PubMed: 8068083]
42. Medzikovic L, Schumacher CA, Verkerk AO, van Deel ED, Wolswinkel R, van der Made I, Bleeker N, Cakici D, van den Hoogenhof MM, Meggouh F, Creemers EE, Remme CA, Baartscheer A, de Winter RJ, de Vries CJ, Arkenbout EK, de Waard V. Orphan nuclear receptor Nur77 affects cardiomyocyte calcium homeostasis and adverse cardiac remodelling. *Sci Rep.* 2015; 5:15404.doi: 10.1038/srep15404 [PubMed: 26486271]
43. Mo FE, Lau LF. The matricellular protein Ccn1 is essential for cardiac development. *Circ Res.* 2006; 99:961–969. DOI: 10.1161/01.RES.0000248426.35019.89 [PubMed: 17023674]
44. Mohammed SF, Storlie JR, Oehler EA, Bowen LA, Korinek J, Lam CS, Simari RD, Burnett JC Jr, Redfield MM. Variable phenotype in murine transverse aortic constriction. *Cardiovasc Pathol.* 2012; 21:188–198. DOI: 10.1016/j.carpath.2011.05.002 [PubMed: 21764606]
45. Montagutelli X. Effect of the genetic background on the phenotype of mouse mutations. *J Am Soc Nephrol.* 2000; 11(Suppl 16):S101–105. [PubMed: 11065339]
46. Ock S, Lee WS, Ahn J, Kim HM, Kang H, Kim HS, Jo D, Abel ED, Lee TJ, Kim J. Deletion of IGF-1 Receptors in Cardiomyocytes Attenuates Cardiac Aging in Male Mice. *Endocrinology.* 2016; 157:336–345. DOI: 10.1210/en.2015-1709 [PubMed: 26469138]
47. Oka T, Xu J, Kaiser RA, Melendez J, Hambleton M, Sargent MA, Lorts A, Brunskill EW, Dorn GW 2nd, Conway SJ, Aronow BJ, Robbins J, Molkentin JD. Genetic manipulation of periostin expression reveals a role in cardiac hypertrophy and ventricular remodeling. *Circ Res.* 2007; 101:313–321. DOI: 10.1161/CIRCRESAHA.107.149047 [PubMed: 17569887]
48. Patten RD, Hall-Porter MR. Small animal models of heart failure: development of novel therapies, past and present. *Circ Heart Fail.* 2009; 2:138–144. DOI: 10.1161/CIRCHEARTFAILURE.108.839761 [PubMed: 19808329]
49. Rapp JP, Wang SM, Dene H. A genetic polymorphism in the renin gene of Dahl rats cosegregates with blood pressure. *Science.* 1989; 243:542–544. DOI: 10.1126/science.2563177 [PubMed: 2563177]
50. Raynolds MV, Bristow MR, Bush EW, Abraham WT, Lowes BD, Zisman LS, Taft CS, Perryman MB. Angiotensin-converting enzyme DD genotype in patients with ischaemic or idiopathic dilated cardiomyopathy. *Lancet.* 1993; 342:1073–1075. DOI: 10.1016/0140-6736(93)92061-W [PubMed: 8105309]
51. Riehle C, Wende AR, Zaha VG, Pires KM, Wayment B, Olsen C, Bugger H, Buchanan J, Wang X, Moreira AB, Doenst T, Medina-Gomez G, Litwin SE, Lelliott CJ, Vidal-Puig A, Abel ED. PGC-1beta deficiency accelerates the transition to heart failure in pressure overload hypertrophy. *Circ Res.* 2011; 109:783–793. DOI: 10.1161/CIRCRESAHA.111.243964 [PubMed: 21799152]
52. Riehle C, Wende AR, Zaha VG, Pires KM, Wayment B, Olsen C, Bugger H, Buchanan J, Wang X, Moreira AB, Doenst T, Medina-Gomez G, Litwin SE, Lelliott CJ, Vidal-Puig A, Abel ED. PGC-1β deficiency accelerates the transition to heart failure in pressure overload hypertrophy. *Circ Res.* 2011; 109:783–793. DOI: 10.1161/CIRCRESAHA.111.243964 [PubMed: 21799152]
53. Rockman HA, Ross RS, Harris AN, Knowlton KU, Steinhilber ME, Field LJ, Ross J Jr, Chien KR. Segregation of atrial-specific and inducible expression of an atrial natriuretic factor transgene in an in vivo murine model of cardiac hypertrophy. *Proc Natl Acad Sci U S A.* 1991; 88:8277–8281. [PubMed: 1832775]
54. Sanford LP, Kallapur S, Ormsby I, Doetschman T. Influence of genetic background on knockout mouse phenotypes. *Methods Mol Biol.* 2001; 158:217–225. DOI: 10.1385/1-59259-220-1:217 [PubMed: 11236659]
55. Sayed D, He M, Yang Z, Lin L, Abdellatif M. Transcriptional regulation patterns revealed by high resolution chromatin immunoprecipitation during cardiac hypertrophy. *J Biol Chem.* 2013; 288:2546–2558. DOI: 10.1074/jbc.M112.429449 [PubMed: 23229551]

56. Sayed D, Yang Z, He M, Pflieger JM, Abdellatif M. Acute targeting of general transcription factor IIB restricts cardiac hypertrophy via selective inhibition of gene transcription. *Circ Heart Fail*. 2015; 8:138–148. DOI: 10.1161/CIRCHEARTFAILURE.114.001660 [PubMed: 25398966]
57. Seong E, Saunders TL, Stewart CL, Burmeister M. To knockout in 129 or in C57BL/6: that is the question. *Trends Genet*. 2004; 20:59–62. DOI: 10.1016/j.tig.2003.12.006 [PubMed: 14746984]
58. Sheikh F, Ouyang K, Campbell SG, Lyon RC, Chuang J, Fitzsimons D, Tangney J, Hidalgo CG, Chung CS, Cheng H, Dalton ND, Gu Y, Kasahara H, Ghassemian M, Omens JH, Peterson KL, Granzier HL, Moss RL, McCulloch AD, Chen J. Mouse and computational models link Mlc2v dephosphorylation to altered myosin kinetics in early cardiac disease. *J Clin Invest*. 2012; 122:1209–1221. DOI: 10.1172/JCI61134 [PubMed: 22426213]
59. Shimano M, Ouchi N, Nakamura K, van Wijk B, Ohashi K, Asaumi Y, Higuchi A, Pimentel DR, Sam F, Murohara T, van den Hoff MJ, Walsh K. Cardiac myocyte follistatin-like 1 functions to attenuate hypertrophy following pressure overload. *Proc Natl Acad Sci U S A*. 2011; 108:E899–906. DOI: 10.1073/pnas.1108559108 [PubMed: 21987816]
60. Small K, Feng JF, Lorenz J, Donnelly ET, Yu A, Im MJ, Dorn GW 2nd, Liggett SB. Cardiac specific overexpression of transglutaminase II (G(h)) results in a unique hypertrophy phenotype independent of phospholipase C activation. *J Biol Chem*. 1999; 274:21291–21296. DOI: 10.1074/jbc.274.30.21291 [PubMed: 10409687]
61. Stapel B, Kohlhaas M, Ricke-Hoch M, Haghikia A, Erschow S, Knuuti J, Silvola JMU, Roivainen A, Saraste A, Nickel AG, Saar JA, Sieve I, Pietzsch S, Muller M, Bogeski I, Kappl R, Jauhainen M, Thackeray J, Scherr M, Bengel FM, Hagl C, Tudorache I, Bauersachs J, Maack C, Hilfiker-Kleiner D. Low STAT3 expression sensitizes to toxic effects of beta-adrenergic receptor stimulation in peripartum cardiomyopathy. *Eur Heart J*. 2017; 38:349–361. DOI: 10.1093/eurheartj/ehw086 [PubMed: 28201733]
62. Szabo Z, Magga J, Alakoski T, Ulvila J, Piuhola J, Vainio L, Kivirikko KI, Vuolteenaho O, Ruskoaho H, Lipson KE, Signore P, Kerkela R. Connective tissue growth factor inhibition attenuates left ventricular remodeling and dysfunction in pressure overload-induced heart failure. *Hypertension*. 2014; 63:1235–1240. DOI: 10.1161/HYPERTENSIONAHA.114.03279 [PubMed: 24688123]
63. Tanaka K, Valero-Munoz M, Wilson RM, Essick EE, Fowler CT, Nakamura K, van den Hoff M, Ouchi N, Sam F. Follistatin like 1 Regulates Hypertrophy in Heart Failure with Preserved Ejection Fraction. *JACC Basic Transl Sci*. 2016; 1:207–221. DOI: 10.1016/j.jacbs.2016.04.002 [PubMed: 27430031]
64. Tanase H, Yamori Y, Hansen CT, Lovenberg W. Heart size in inbred strains of rats. Part 1. Genetic determination of the development of cardiovascular enlargement in rats. *Hypertension*. 1982; 4:864–872. DOI: 10.1161/01.HYP.4.6.864 [PubMed: 6216212]
65. Tomita H, Hagaman J, Friedman MH, Maeda N. Relationship between hemodynamics and atherosclerosis in aortic arches of apolipoprotein E-null mice on 129S6/SvEvTac and C57BL/6J genetic backgrounds. *Atherosclerosis*. 2012; 220:78–85. DOI: 10.1016/j.atherosclerosis.2011.10.020 [PubMed: 22078246]
66. Tomita H, Zhilicheva S, Kim S, Maeda N. Aortic arch curvature and atherosclerosis have overlapping quantitative trait loci in a cross between 129S6/SvEvTac and C57BL/6J apolipoprotein E-null mice. *Circ Res*. 2010; 106:1052–1060. DOI: 10.1161/CIRCRESAHA.109.207175 [PubMed: 20133902]
67. Troncoso R, Ibarra C, Vicencio JM, Jaimovich E, Lavandero S. New insights into IGF-1 signaling in the heart. *Trends Endocrin Met*. 2014; 25:128–137. DOI: 10.1016/j.tem.2013.12.002
68. Wang H, Kwak D, Fassett J, Liu X, Yao W, Weng X, Xu X, Xu Y, Bache RJ, Mueller DL, Chen Y. Role of bone marrow-derived CD11c+ dendritic cells in systolic overload-induced left ventricular inflammation, fibrosis and hypertrophy. *Basic Res Cardiol*. 2017; 112:25. doi: 10.1007/s00395-017-0615-4 [PubMed: 28349258]
69. Wang JG, Staessen JA. Genetic polymorphisms in the renin-angiotensin system: relevance for susceptibility to cardiovascular disease. *Eur J Pharmacol*. 2000; 410:289–302. DOI: 10.1016/S0014-2999(00)00822-0 [PubMed: 11134677]
70. Wang RH, He JP, Su ML, Luo J, Xu M, Du XD, Chen HZ, Wang WJ, Wang Y, Zhang N, Zhao BX, Zhao WX, Shan ZG, Han J, Chang C, Wu Q. The orphan receptor TR3 participates in angiotensin

- II-induced cardiac hypertrophy by controlling mTOR signalling. *EMBO Mol Med.* 2013; 5:137–148. DOI: 10.1002/emmm.201201369 [PubMed: 23197407]
71. Welch S, Plank D, Witt S, Glascock B, Schaefer E, Chimenti S, Andreoli AM, Limana F, Leri A, Kajstura J, Anversa P, Sussman MA. Cardiac-specific IGF-1 expression attenuates dilated cardiomyopathy in tropomodulin-overexpressing transgenic mice. *Circ Res.* 2002; 90:641–648. DOI: 10.1161/01.RES.0000013780.77774.75 [PubMed: 11934830]
72. Xu W, Barrientos T, Mao L, Rockman HA, Sauve AA, Andrews NC. Lethal Cardiomyopathy in Mice Lacking Transferrin Receptor in the Heart. *Cell Rep.* 2015; 13:533–545. DOI: 10.1016/j.celrep.2015.09.023 [PubMed: 26456827]
73. Yan G, Zhu N, Huang S, Yi B, Shang X, Chen M, Wang N, Zhang GX, Talarico JA, Tilley DG, Gao E, Sun J. Orphan Nuclear Receptor Nur77 Inhibits Cardiac Hypertrophic Response to Beta-Adrenergic Stimulation. *Mol Cell Biol.* 2015; 35:3312–3323. DOI: 10.1128/MCB.00229-15 [PubMed: 26195821]
74. Zhao S, Wu HF, Xia WL, Chen XJ, Zhu SS, Zhang SJ, Shao YF, Ma WZ, Yang D, Zhang JN. Periostin expression is upregulated and associated with myocardial fibrosis in human failing hearts. *J Cardiol.* 2014; 63:373–378. DOI: 10.1016/j.jjcc.2013.09.013 [PubMed: 24219836]
75. Zhu H, Zhang J, Shih J, Lopez-Bertoni F, Hagaman JR, Maeda N, Friedman MH. Differences in aortic arch geometry, hemodynamics, and plaque patterns between C57BL/6 and 129/SvEv mice. *J Biomech Eng.* 2009; 131:121005.doi: 10.1115/1.4000168 [PubMed: 20524728]

Fig. 1. Development of contractile dysfunction in C57BL/6J and 129S1/SvImJ mice following TAC

Time course of ejection fraction and endsystolic volume determined by echocardiography in C57BL/6J (a, b) and 129S1/SvImJ (c, d) mice undergoing Sham surgery or TAC surgery. Heart weight/body weight (HW/BW) and lung weight/body weight (LW/BW) ratio in C57BL/6J (e) and 129S1/SvImJ (f) mice ten or twelve weeks following Sham or TAC surgery, respectively. Trans-stenotic peak pressure gradient at site of aortic constriction in TAC operated C57BL/6J and 129S1/SvImJ mice relative to their sham operated littermates (g). Clip diameter for TAC: C57BL/6J mice: 32G, 129S1/SvImJ mice: 34G. T-test: * $P < 0.05$ vs. Sham or as indicated. 2-way ANOVA: \$: Effect of treatment.

Fig. 2. Cardiac remodeling in C57BL/6J and 129S1/SvImJ mice following TAC

Myocardial gene expression of α -MHC, β -MHC and ANP (a, b) and histological analysis of frozen heart sections in C57BL/6J and 129S1/SvImJ mice ten or twelve weeks following Sham or TAC surgery, respectively (c-h). The following parameters were analyzed histologically using the staining/antibody in brackets: Cardiomyocyte size (WGA), myocardial fibrosis (Trichrome), capillary density (CD31), cell proliferation (Ki-67), macrophage infiltration (MAC3), and apoptosis (TUNEL). Clip diameter for TAC: C57BL/6J mice: 32G, 129S1/SvImJ mice: 34G. T-test: * $P < 0.05$ vs. Sham or as indicated. 2-way ANOVA: \$: Effect of treatment, \$: Effect of genotype, \$: Interaction.

Fig. 3. Differential gene expression in C57BL/6J and 129S1/SvImJ mice following TAC
 (a) Venn Diagram showing the overlap in differential gene expression between the C57BL/6J and 129S1/SvImJ mice with and without TAC. (b) GO Term Enrichment Analysis depicting the top 5 Toxicity Networks ranked according to $-\log(P\text{-value})$ for C57BL/6J strain. Differentially-expressed genes responsible for populating each toxicity network were hierarchically clustered to examine overlapping gene annotations, with heatmap illustrating the fold-change of all genes reaching significance for C57BL/6J mice. *Genes reaching significance (Fisher's exact test $P < 0.05$, $|\text{Fold-change}| > 1.5$) for 129S1/SvImJ TAC vs. 129S1/SvImJ Sham. (c) Toxicity Network "Cardiac Hypertrophy" bar graph depicting the RNA expression fold-changes of the differentially expressed genes by 129S1/SvImJ (red) and C57BL/6J (black). Differential gene expression was considered significant when $\text{Fold-Change} > 1.5$ and $P < 0.05$ (Fisher's exact test) For detailed gene information and pathway analysis please refer to Tables S8 and S9.

Fig. 4. Effect of TAC on Global Transcriptional Changes

Hierarchical clustering and Heatmap visualization of statistically-significant changes (Fisher's exact test $P < 0.05$) in gene expression in C57BL/6J TAC + HF vs. C57BL/6J Sham + NF (left figure) and 129S1/SvImJ TAC + HF vs. 129S1/SvImJ Sham + NF (right figure). Human HF and NF microarray data were filtered according to differentially expressed mouse homologues (Fisher's exact test $P < 0.05$). C57BL/6J: $n=5$; 129S1/SvImJ: $n=4$; Human: $n=5$. For gene list and changes please refer to Table S11.

Fig. 5. C57BL/6J More Closely Depicts the Failing Human Heart

(a) Venn Diagram depicting the overlap of differential gene expression between Human heart failure vs. non-failing hearts, C57BL/6J mouse TAC vs. Sham, and 129S1/SvImJ mouse TAC vs. Sham, with (b) Bar graph depicting the RNA expression fold-changes of the differentially expressed genes that are co-expressed among all three comparisons. (c) Top 10 Putative Upstream Regulators common to C57BL/6J TAC vs. Sham and Human HF vs. NF comparisons, ranked according to Z-Score among the statistically-significant molecules. (d) Top upstream regulator analysis using Ingenuity Pathway Analysis (IPA) for Human heart failure (n = 5) and C57BL/6J TAC vs. Sham (n = 5) identifies angiotensin (AGT) as a putative nodal regulator. *Differential gene expression was considered significant when Fold-Change > 1.5 and *P*<0.05 (Fisher's exact test). For detailed gene information and pathway analysis please refer to Table S12.

Fig. 6. Transcriptional Comparisons Analysis

Gene set enrichment analysis between C57BL/6J TAC vs. Sham (n = 5) and Human HF vs. NF (n = 5). (a) Top 10 Gene Ontology (GO) Term enriched canonical pathways sorted according to Bonferroni-adjusted P-value. (b) A bar graph of the RNA expression fold-change of the differentially expressed genes that constitute the top upstream pathway, IGF-1 Signaling. IPA was used on differential gene expression achieving Fold-Change > 1.5 and $P < 0.05$ (Fisher's exact test). For detailed pathway information please refer to Table S13.

Table 1

Selected genes that were commonly regulated in C57BL/6J and 129S1/SvImJ mice in response to TAC and that have been linked to the pathogenesis of heart failure previously.

Gene symbol	Gene name	Fold change (C57BL/6J)	Fold Change (129S1/SvImJ)	Predicted function
<i>Postn</i>	Periostin	16.7	3.7	Maladaptive
<i>Tgm2</i>	Transglutaminase 2	1.6	1.7	Maladaptive
<i>Fstl1</i>	Follistatin like 1	4.1	2.0	Adaptive
<i>Stat3</i>	Signal transducer and activator of transcription 3	2.0	1.7	Adaptive

Predicted function (adaptive or maladaptive) is based on publications listed in Table S10. List includes genes with at least |1.5-fold| regulation and $P < 0.05$ by Fisher's exact test.

Table 2

Selected genes that were uniquely regulated in C57BL/6J or 129S1/SvImJ mice in response to TAC and that have been linked to the pathogenesis of heart failure previously.

Strain	Gene symbol	Gene name	Fold change	Predicted function
C57BL/6J	<i>Thbs4</i>	thrombospondin 4	9.1	Adaptive
C57BL/6J	<i>Comp</i>	cartilage oligomeric matrix protein	7.4	Adaptive
C57BL/6J	<i>Frzb</i>	frizzled-related protein	5.4	Adaptive
C57BL/6J	<i>Ctgf</i>	connective tissue growth factor	5.0	Maladaptive
C57BL/6J	<i>Nox4</i>	NADPH oxidase 4	4.3	Maladaptive
C57BL/6J	<i>Wisp2</i>	WNT1 inducible signaling pathway protein 2	4.3	Adaptive
C57BL/6J	<i>Lox</i>	lysyl oxidase	4.0	Maladaptive
C57BL/6J	<i>Fn1</i>	fibronectin 1	3.8	Maladaptive
C57BL/6J	<i>Tgfb2</i>	transforming growth factor beta 2	3.5	Maladaptive
C57BL/6J	<i>Fbln2</i>	fibulin 2	3.3	Maladaptive
C57BL/6J	<i>Stip1</i>	secreted frizzled related protein 1	2.7	Adaptive
C57BL/6J	<i>Tgfb3</i>	transforming growth factor beta 3	2.4	Maladaptive
C57BL/6J	<i>Pcolce</i>	procollagen C-endopeptidase enhancer	2.2	Maladaptive
C57BL/6J	<i>Tgfr2</i>	transforming growth factor beta receptor 2	2.0	Adaptive
C57BL/6J	<i>Grk5</i>	G protein-coupled receptor kinase 5	2.0	Maladaptive
C57BL/6J	<i>Timp2</i>	TIMP metalloproteinase inhibitor 2	1.8	Adaptive
C57BL/6J	<i>Ctsa</i>	cathepsin A	1.7	Maladaptive
C57BL/6J	<i>Tgfr1</i>	transforming growth factor beta receptor 1	1.7	Maladaptive
C57BL/6J	<i>Rbfox1</i>	RNA binding protein, fox-1 homolog 1	-1.7	Maladaptive
C57BL/6J	<i>Foxo3</i>	forkhead box O3	-1.8	Maladaptive
C57BL/6J	<i>Maob</i>	monoamine oxidase B	-2.4	Adaptive
C57BL/6J	<i>Rgs2</i>	regulator of G-protein signaling 2	-2.7	Maladaptive
129S1/SvImJ	<i>P4ha1</i>	prolyl 4-hydroxylase subunit alpha 1	1.7	Maladaptive

Predicted function (adaptive or maladaptive) is based on publications listed in Table S10. List includes genes with at least |1.5-fold| regulation and $P < 0.05$ by Fisher's exact test.