The year 2013 has witnessed exciting advances in cardiovascular research exemplified by discovery leveraging genomics, proteomics and metabolomics platforms, genome editing, translation in individualized disease modeling, and application of regenerative therapies. New knowledge generated through integrative analysis has informed the understanding of molecular mechanisms that can now be validated and exploited to expedite management of biological processes.

The Functional Genomics and Translational Biology Council from the American Heart Association (www.my.americanheart.org/fgtbcouncil) has the mission of advancing new discoveries in the fields of genetics, omics technologies, and translational biology and facilitate their application in cardiovascular health and disease. By creating a multidisciplinary collaborative environment, this Council integrates scientific knowledge from molecules to populations to contribute to the global American Heart Association goal of building healthier lives, free of cardiovascular diseases and stroke.

With input from the Early Career Committee of the Council on Functional Genomics and Translational Biology, we considered many outstanding articles and selected 10 advances published during 2013. In this Special Report, we summarize some relevant articles related to each of those advances and highlight their significance in the cardiovascular field.

Cardiac hypertrophy is an increasingly prevalent condition because of population aging with limited therapeutic options to prevent its progression to heart failure. Loffredo et al1 hypothesized that loss of systemic factors may be responsible for the stiffening and enlargement of the ventricles and thereby a therapeutic target for the treatment of systolic heart failure. Using a model of parabiosis (surgically linking the systemic circulation of 2 animals), they demonstrated that exposure to a young circulatory environment can reverse age-induced cardiac hypertrophy in old mice. Proteomic analysis of old and young adult plasma enabled identification of the growth differentiation factor 11, a member of the transforming growth factor-β superfamily, as a factor with decreasing blood levels in aging mice. Growth differentiation factor 11 was found to prevent cardiac hypertrophy in vitro and reverse age-related hypertrophy in vivo, with restoration of circulating levels of growth differentiation factor 11 in old mice leading to decreased heart size. In addition, growth differentiation factor 11 supplementation led to reduced cardiomyocyte size, downregulation of cardiac hypertrophy markers (atrial natriuretic peptide and brain-type natriuretic peptide), and increased expression of sarco(endoplasmic reticulum Ca2+ ATPase (SERCA2), all markers of improved structure and function. Thus, this study identifies a cardiac rejuvenating factor naturally present in the systemic circulation of young individuals opening up exciting possibilities for the treatment of age-related cardiac hypertrophy.

Congenital heart disease (CHD) is one of the most common heterogeneous birth defects. Large-scale genetic efforts to identify de novo mutations causing CHD were lacking. Zaidi et al2 demonstrated a striking application of the advances in high-throughput sequencing technologies by performing exome sequencing to compare the incidence of de novo mutations in parent-offspring trios of 362 severe CHD cases and 264 controls. De novo mutation rates in CHD cases and controls were not different. The authors hypothesized that genes causing CHD should be expressed highly in the developing heart. The authors used RNA-sequencing data from mouse embryos at day E14.5 to segregate 4169 genes with highest heart expression in the top quartile for further analyses. As compared with controls, CHD cases were 7.5x more likely to show damaging (premature termination, splice site, and frameshift) mutations in genes expressed in the developing heart. Similar results were obtained when genes were partitioned across a range of expression thresholds, and mouse embryos at day E9.5 were used. Overall, de novo mutations were shown to account for ≈10% of severe CHD cases. Finally, the authors conducted pathway analysis to discover a significant association in the histone H3 lysine 4 methylation pathway or ubiquitination of H2BK120, which is required for histone H3 lysine 4 methylation. Further mechanistic studies are needed to see whether these initial genetic findings can help us improve patient care or yield insight into why some patients with CHD with similar anatomic defects respond better to surgery than others.

Exome sequencing in a well-phenotyped family with myocardial infarction by Erdmann et al3 led to the discovery of rare variants that disrupt nitric oxide signaling in platelets leading to accelerated thrombus formation. The family where 22 members had myocardial infarction before the age of 60 years was identified as part of the German Myocardial Infarction Family Study. Sequencing 3 distantly related family members,
During the past year, genome editing technology has blossomed allowing functional studies of known and newly discovered gene variants. In a thorough work, Ding et al discovered a tissue-specific enhancer by conducting an unbiased chromatin mapping efforts have indicated that common genetic variation associated with complex traits often mark regulatory hotspots in the genomic DNA. Bauer et al discovered a tissue-specific enhancer by conducting follow-up mechanistic studies of common genetic variants in the BCL11A gene associated with elevated fetal hemoglobin levels. The authors noted by running allele-specific analyses that the genetic variation within this enhancer impairs binding of transcription factor, BCL11A expression, and fetal hemoglobin level. To study the enhancer potential, the authors cloned the 12-kb regulatory sequence identified in intron 2 of the BCL11A gene in a mouse transgenic reporter assay. The reporter gene expression was confined largely to the fetal liver (the site of erythropoiesis), as opposed to the endogenous BCL11A, which showed abundant expression in the central nervous system. The authors tested whether the enhancer was required for BCL11A expression in mouse erythroleukemia cell line. They used transcription activator–like effector nucleases (TALEN) to modulate the activity of the enhancer sequence critical for expression of BCL11A. They were able to confirm the erythroid specificity of the enhancer by demonstrating impaired BCL11A production in the murine erythroid cell line with no effect on BCL11A production in the lymphocyte cell line. With further translational work, one can hope that the BCL11A enhancer may serve as a drug target to treat fatal hemoglobinopathies such as sickle cell disease.

During the past year, genome editing technology has blossomed allowing functional studies of known and newly discovered gene mutations. In a thorough work, Ding et al leveraged a TALEN genome editing system together with human somatic or pluripotent stem cells to generate lines harboring mutations in several genes of interest while having isogenic lines that can be used as background-matched controls. Taking advantage of the plasticity of pluripotent cells, the authors differentiated control and gene-edited lines into lineages where the function of each gene is more relevant and performed individual phenotypic studies validating or discovering new mutation roles. Specifically, this study confirms the critical role of the apolipoprotein B for human hepatitis C virus replication through TALEN–directed truncation of the APOB gene. In addition, differentiation of human pluripotent lines (controls and specifically mutated in sortilin) into hepatocyte-like cells, white adipocytes, and motor neurons led to functional results, suggesting that sortilin is involved in maintaining low cholesterol levels, optimizing insulin sensitivity and regulating neural programmed cell death. Of note, some of these results contradict previous reports described in mouse models, highlighting the interspecies variability that can be bypassed thanks to this cutting edge technology. Also, by recapitulating allele variants found in the clinic in an in vitro system, the pathological phenotype observed in several patients with AKT2 and peripilin mutations could be mechanistically justified at the cellular level. Finally, this study seeks deeper insights into the after effects of TALEN gene editing, concluding that off-target mutagenesis is uncommon although residual single nucleotide variants not directly attributable to this technology are found. Overall, this work presents broad evidence of a new generation of rapidly evolving molecular tools that enable stringently controlled functional studies to advance genomic research.

Numerous patients with heart failure have a poor clinical outcome, despite receiving optimal therapy. In a phase II, randomized, double-blind, placebo-controlled clinical trial, Zscho et al investigated the long-term effectiveness and safety of a gene therapy strategy based on an adeno-associated virus 1 vector carrying the sarcoplasmic reticulum ATPase pump (AAV1/SERCA2a) in 39 patients with advanced heart failure. Patients received a single intracoronary infusion of AAV1/SERCA2a (low, middle, or high dose) or placebo and were followed up for 3 years. Those on the high dose had fewer recurrent cardiovascular events compared with placebo (equivalent to an 82% reduction in risk). Patients on the low or mid dose had a similar number of recurrent events as the placebo group, although these were delayed by treatment. No safety concerns were identified and analysis of myocardial biopsies showed that a single infusion of AAV1/SERCA2a at the high dose was sufficient to generate robust expression of the transgene for ≥31 months. Despite the relatively small sample size, this study is noteworthy as it describes the long-term consequences of cardiac gene transfer with an AAV1 vector. The findings so far provide encouraging initial evidence that AAV1/SERCA2a therapy may have long-term therapeutic benefits in patients with heart failure and pave the way for other gene therapy trials using AAV1 vectors in heart failure and other myocardial diseases.

Hypertrophic cardiomyopathy (HCM) is caused by mutations in components of the cardiac sarcomere (such as the myosin heavy chains), although treatment strategies that target these underlying abnormalities directly are lacking. Jiang et al tested the hypothesis that selective reduction of mutant myosin heavy chain transcripts in the heart would restore sarcomere function and prevent HCM. They developed an RNA silencing approach based on adeno-associated viral vector delivery.
of RNAi constructs that allowed allele-specific repression of mutant myosin heavy chain transcripts (while maintaining wild-type expression), in transgenic mice heterozygous for the HCM-causing myosin heavy chain *Myh6* R403Q mutation. A reduction in the levels of mutant transcripts in these mice by only 28% was sufficient to prevent myocyte disarray, myocardial fibrosis, and hypertrophy characteristic of HCM for ≥26 months, suggesting that unequal expression of mutant and wild-type myosin heavy chain transcripts in human HCM may contribute to the variable penetrance and severity of this disease. From a practical perspective, the authors also showed that an RNAi construct that targeted a nearby single nucleotide polymorphism that distinguished between wild-type and mutant alleles could also repress expression of the mutant allele, potentially avoiding the need to develop thousands of different RNAi molecules specific for each unique HCM mutation. This study highlights the possibility that gene therapy strategies based on allele-specific silencing may be able to slow the onset and progression of HCM and other genetic cardiomyopathies.

Although several genes have been associated with familial HCM, the underlying mechanism of this disease remains unclear. Lan et al. leveraged induced pluripotent stem cell (iPSC) technology to generate pluripotent lines from HCM patients containing a mutation in the β-myosin heavy chain (*MYH7*) gene as well as healthy relatives. This iPSC platform provides an unlimited source of patient and control-derived cells to be used for phenotypic studies bypassing the limited availability of cardiac samples. In vitro differentiation and characterization of patient and control-derived lines revealed that *MYH7*-iPSCs recapitulate relevant features of HCM, including enlarged cardiomyocytes, increased myofibril content with disorganized sarcomeric structures, as well as elevated expression of several disease markers. In addition, the calcineurin-NFAT axis was identified as an important player in the development of HCM in the context of the studied mutation. Based on arrhythmic potentials and abnormal contractile activity observed in HCM-derived cardiomyocytes, calcium dynamics were studied as a possible disease mechanism. The authors found several lines of evidence, suggesting that calcium handling might be the underlying cause of hypertrophy: (1) abnormal calcium transients occur before cellular enlargement, (2) intracellular calcium levels are higher in diseased cells, and (3) calcium release from the sarcoplasmic reticulum is reduced in HCM-iPSCs. Moreover, treatment of calcium dysregulation with L-type calcium blocker verapamil ameliorated pathological features such as cellular hypertrophy, calcium-handling abnormalities, and arrhythmia. Finally, patient-specific cardiomyocytes were tested as screening platform for drugs used in the treatment of HCM revealing variable results at the single cell level depending on the drug target. Thus, this study provides a new mechanistic explanation linking calcium imbalance and HCM and establishes patient-derived iPSCs as a valuable tool for in vitro phenotypic analysis as well as drug-testing platform in cardiovascular disease.

Using different approaches, studies by Arora et al. and Hinkel et al. illustrated the future promise of miRNA-based therapies for the treatment of cardiovascular disease. Arora et al. sought to uncover the mechanism underlying the association between blood pressure and rs5068 (A/G), a variant located in the 3′untranslated region of *NPPA*, which encodes atrial natriuretic peptide. They demonstrated that the presence of the rs5068 G allele was sufficient to prevent binding of miR-425, a miRNA found in both atria and ventricles, resulting in increased *NPPA* expression and higher atrial natriuretic peptide levels. Of particular interest, this work illustrated that genetic variants with modest effects on clinical traits may have substantial effects on physiological responses. This study provides new mechanistic insight into the regulation of blood pressure and salt homeostasis and offers a potential new therapeutic miRNA target for treatment of hypertension. Hinkel et al. tested the efficacy of a novel anti-miR therapy for myocardial injury by investigating whether delivery of a locked nucleic acid–modified antisense miR-92a (LNA-92a) could augment recovery in a pig ischemia–reperfusion model. They demonstrated that a single infusion of LNA-92a reduced expression of miR-92a in the heart, reduced infarct size, improved left ventricular function and provided beneficial anti-inflammatory, antiapoptotic, and proangiogenic effects, consistent with anti–miR-92a studies in mice. Local catheter-based delivery enabled use of a submaximal dose of LNA-92a that minimized off-target effects while maintaining efficacy. This study provides the first evidence that inhibition of miRNAs can provide a functional benefit in a large animal model of cardiac ischemia–reperfusion injury and contributes important preclinical data for the development of miRNA-based therapies for patients receiving interventional treatment after myocardial infarction.

The gut microbiome plays an important role in drug metabolism, acting as a modifying factor that affects efficacy and toxicity. Haiser et al. examined this phenomenon by investigating the molecular mechanism underlying inactivation of the cardiac drug digoxin by the gut bacterium *Eggerthella lenta*. Digoxin is known to be reduced in vivo, which decreases its affinity for its target, the Na+/K+ ATPase, in cardiac myocytes. This reduction can be mitigated in vivo by coadministration of broad-spectrum antibiotics and recapitulated in vitro by *E. lenta*. Using RNA sequencing in type strain *E. lenta* cultures treated with digoxin, the authors identified a 2-gene operon (*cgr*) containing 2 reductases (*cgr1* and *cgr2*) potentially responsible for metabolizing the drug. This operon was not present in other nonmetabolizing *E. lenta* strains, and its upregulation (>100-fold in the presence of digoxin) was repressed in high arginine culture conditions. In addition, expression levels of *cgr* in microbiome samples from healthy individuals correlated with their capacity to reduce and inactivate digoxin. Beyond characterization of the intrinsic reduction properties of the *E. lenta* type strain, the authors described the modulation of digoxin inactivation by coculture of the type strain with a heterogeneous fecal microbiome (by promoting growth of *E. lenta* through synergistic microbial interaction) as well as inhibition of digoxin inactivation by high protein diet (proposed to repress expression of *cgr*). Overall, this study highlights a new compartment in drug metabolism models that may influence systemic drug levels and help guide dosage regimes.
Appendix
Early Career Members of the Functional Genomics and Translational Biology Council
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Disclosures
Dr Arora is named as co-inventor on a patent application relating to the use of miRNAs for the treatment of hypertension and other disorders. The other authors report no conflicts.

References

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