Modulation of Mitochondrial Proteome and Improved Mitochondrial Function by Biventricular Pacing of Dyssynchronous Failing Hearts

Summary: One of the limitations of cardiac resynchronization therapy is that 20% to 30% of the patients demonstrate little clinical benefit, if any at all. We believe that insight regarding the molecular mechanisms that are activated by cardiac resynchronization therapy may provide candidate targets that identify responders to this therapy and that proteomics could help in pinpointing the key proteins in this process and could narrow down the search for pathways that are dysregulated in nonresponders. Additionally, the study explores what the beneficial pathways activated by cardiac resynchronization therapy are in a well-established in vivo model.

Conclusions: Cardiac resynchronization therapy potently affects both the mitochondrial proteome and the performance associated with improved cardiac function.

Comprehensive Analysis of Genomic Variation in the LPA Locus and Its Relationship to Plasma Lipoprotein(a) in South Asians, Chinese, and European Caucasians

Summary: Increased cardiovascular disease risk has been associated with plasma lipoprotein(a) (Lp(a)) levels, though somewhat inconsistently, limiting implementation in common clinical practice. Circulating concentrations of Lp(a) are largely under the control of genetic variation in the LPA gene due to the cumulative effects of multiple rare and common genetic variants. A common copy number variation in LPA, created by a variable number of exons coding for kringle IV type 2 domains, is known to affect Lp(a) concentration but, until recently, has not been measurable in cohorts with limited quantities of genomic DNA available. We demonstrate that a correlation exists between single-nucleotide polymorphisms in the LPA locus and kringle IV type 2 copy number and that both LPA single-nucleotide polymorphisms and kringle IV type 2 copy number contribute to Lp(a) concentration and, thus, should be measured in future studies of Lp(a). In agreement with previous work, the relationship between genomic variation in LPA and Lp(a) concentration appears to vary among ethnicities. Additional genetic variants that are too rare, have effects too small to be detected in the SHARE sample, or were not queried in this study, play a greater role in South Asians and Chinese. Further identification of rare and common variants is required in these and other ethnicities. Finally, our work shows that future Mendelian randomization studies investigating Lp(a) would benefit from including both LPA single-nucleotide polymorphisms and kringle IV type 2 copy number, as the extent of Lp(a) concentration explained is larger using both types of variation.

Conclusions: LPA SNPs are in linkage disequilibrium with KIV-2 copy number, but KIV-2 copy number explains an increment in plasma Lp(a) variation over SNPs alone. Thus, both SNPs and KIV-2 copy number should be included in future genetic epidemiology studies of Lp(a).2

Altered Hepatic Gene Expression Profiles Associated With Myocardial Ischemia

Summary: Acute coronary syndrome (ACS) is accompanied by systemic changes in inflammation, coagulation, and metabolism, which may affect the outcome and prognosis of ACS. These systemic reactions are not explained by cardiac events alone. Several lines of evidence suggest that patients with fatty liver disease have a high risk of developing cardiovascular diseases, and it is possible to speculate that the liver is involved in a systemic reaction that modifies the pathogenesis of ACS. However, the relation between liver and myocardial ischemia in the acute ischemic phase has not been elucidated so far. In this investigation, we simultaneously analyzed the gene expression profiles of the liver and heart during acute myocardial ischemia in mice and observed the presence of humoral factors that intervened between the heart and liver. These humoral factors were released from the heart and influenced the liver to secrete important tissue remodeling factors. One of these humoral factors, osteopontin, a widely expressed glycoprotein, was increased in the ischemic heart and altered the gene expression of hepatocytes to produce important tissue remodeling factors (such as vascular endothelial growth factor-A). Our observations suggest that hepatic gene expression is potentially regulated by humoral factors of cardiac origin provoked by myocardial ischemia, and we provide direct evidence that the liver is involved in a systemic reaction that accompanies ACS. Our findings provide potential new insights into the pathophysiology of ACS.

Conclusions: Hepatic gene expression is potentially regulated by cardiac humoral factors under myocardial ischemia. These results provide new insights into the pathophysiology of acute coronary syndrome.

Heart Failure–Associated Changes in RNA Splicing of Sarcomere Genes

Summary: Changes in gene expression accompany and contribute to heart failure. These gene expression changes have been documented
at the whole-gene level using microarray-based, genome-wide expression profiling approaches. However, global changes in mRNA splicing in heart failure have not been previously studied. Here, we use a microarray that individually measures expression of each known or putative exon and reverse transcription polymerase chain reaction assays to examine splicing changes that accompany heart failure. We found that broad changes in mRNA splicing occur in the failing heart. Many of these changes were indicative of decreased efficiency of mRNA splicing, and among the affected genes, there were several sarcomere genes. These splicing changes also were observed in hypertrophied myocardium with preserved systolic function, suggesting that the splicing changes may occur before the onset of overt heart failure. The splicing changes were characteristic of diseased myocardium. A prediction model using the splicing of 3 sarcomere genes discriminated failing from nonfailing hearts with 98% accuracy, providing proof of principle that splicing-based biomarkers may provide diagnostic and prognostic information on patients with heart disease. Future studies will be needed to prospectively evaluate the value of splicing-based myocardial biomarkers in individualizing therapy in heart failure. Future work also will be needed to determine whether changes in RNA splicing are functionally important in the pathogenesis of heart disease and whether targeted modulation of splicing is a useful therapeutic strategy for heart failure.

Conclusions: Our data indicate that mRNA splicing is broadly altered in human heart disease and that patterns of aberrant RNA splicing accurately assign samples to control or disease classes.4

Associations of Lipoprotein Lipase Gene Polymorphisms With Longitudinal Plasma Lipid Trends in Young Adults: The Coronary Artery Risk Development in Young Adults (CARDIA) Study

Summary: Lipoprotein lipase plays a key role in the metabolism of lipoproteins and regulation of triglyceride and high-density lipoprotein cholesterol levels. Based on the data from 2045 African American men and women aged 21-16 years of age at baseline, a subsample of 371 men and women at follow-up visits at ages 7 and 11 examinations, we detected multiple variants in the lipoprotein lipase gene that are associated with interindividual differences in triglycerides and high-density lipoprotein cholesterol and with longitudinal changes in the 2 lipids during 20 years of life span. Our findings may provide useful information in risk prediction and intervention for lipid disorders and coronary heart disease.

Conclusions: Our data suggest that aging interacts with LPL gene variants to influence the longitudinal lipid variations, and there is population-related heterogeneity in the longitudinal associations.5

Genomic Variation Associated With Mortality Among Adults of European and African Ancestry With Heart Failure: The Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium

Summary: This study investigated 2526 incident heart failure (HF) cases of European ancestry and 466 incident HF cases of African ancestry for an association between genome-wide variation and all-cause mortality. One variant in the CKLF-like MARVEL transmembrane domain containing 7 (CMTM7) genes was significantly associated with all-cause mortality in individuals of European ancestry with HF. CMTM7 is 1 of several chemokine-like factor genes clustered in the same region on chromosome 3p22. These results suggest that chemokines may play a role in survival of patients with HF. No genomic variation was significantly associated with mortality in individuals with HF of African ancestry. Future studies of this type may identify genes that lead to an improved understanding of HF pathophysiology and treatment of the disease. These findings warrant additional investigation, including replication, in other studies of HF.

Conclusions: This study identified a novel locus associated with all-cause mortality among individuals of European ancestry with HF. This finding warrants additional investigation, including replication, in other studies of HF.6

A Common Variant at Chromosome 9P21.3 Is Associated With Age of Onset of Coronary Disease But Not Subsequent Mortality

Summary: The chromosome 9p21.3 locus has been identified in multiple studies as being strongly associated with the risk of developing coronary artery disease (CAD). However, once CAD is established, the effect of this locus on the progression of disease remains largely unknown. This study investigated the association between the chromosome 9p21.3 single-nucleotide polymorphism rs1333049 and clinical outcomes in 2 cohorts of patients with CAD. Although no association was identified between rs1333049 and all-cause mortality or subsequent hospital admission for a cardiovascular disease event in both patient cohorts, participants carrying the high-risk CC genotype on average developed CAD 2 to 5 years earlier than the other patient groups. This earlier age of onset of CAD in patients with the high-risk genotype, if confirmed, may have important clinical implications.

Conclusions: The chr9p21.3 polymorphism rs1333049 was associated with an earlier age of disease onset in 2 coronary disease cohorts but not with poorer clinical outcome in either cohort.7

CREB1 Is a Strong Genetic Predictor of the Variation in Exercise Heart Rate Response to Regular Exercise: The HERITAGE Family Study

Summary: Regular physical activity is a cornerstone of a heart-healthy lifestyle. Exercise training improves cardiac function and several cardiovascular disease risk factors, including ability to perform physical tasks at a given workload with a lower heart rate. However, the cardiovascular benefits of regular physical activity are not equally distributed among individuals, because some exhibit marked improvements whereas others may show little or no changes. Our previous work has shown that interindividual variation in responsiveness to training aggregates in families. Herein, we show that DNA sequence variation in the CAMP-responsive element-binding protein 1 gene locus is a strong genetic predictor of variation in exercise training–induced changes in submaximal exercise heart rate, explaining about 5% of the total variance. Better understanding of the predictors of high and low responsiveness to regular physical activity has physiological, clinical, and public health relevance. Such information would help to identify those individuals who would derive the greatest health benefits from exercise training as well as patients who would need other therapeutic options (diet and medication) to support a physically active lifestyle.

Conclusions: Our data suggest that functional DNA sequence variation in the CREB1 locus is strongly associated with AHRS0 and explains a considerable proportion of the quantitative trait locus variance. However, at least 5 additional SNPs seem to be required to fully account for the original linkage signal.8

Association of Genome-Wide Variation With the Risk of Incident Heart Failure in Adults of European and African Ancestry: A Prospective Meta-Analysis From the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium

Summary: Genetic factors contribute to heart failure (HF) onset and, to date, most approaches to identify genetic variants associated with...
HF risk have relied on candidate genes. No large-scale genome-wide investigation of HF risk has been published. We investigated the association of \(\sim 2.5\) million single-nucleotide polymorphisms with incident HF by meta-analyzing data from 4 community-based prospective cohorts: the Atherosclerosis Risk in Communities Study, the Cardiovascular Health Study, the Framingham Heart Study, and the Rotterdam Study. Among 20 926 European-ancestry participants with 2526 incident HF events, we identified 1 locus with a single-nucleotide polymorphism, whose probability value \(4 \times 10^{-5}\) exceeded the genome-wide statistical significance threshold of \(5 \times 10^{-7} \) and was associated with a 53% increase in HF risk. Among 2895 African-ancestry participants who had 466 incident events, we identified 1 locus with a single-nucleotide polymorphism, whose probability value \(6.7 \times 10^{-5}\) exceeded the genome-wide statistical significance and was associated with a 46% increase in HF risk. We identified an additional 14 loci in European-ancestry and African-ancestry participants that were marked by high-signal single-nucleotide polymorphisms with a probability value \(< 1 \times 10^{-8}\). For most loci, risk estimates were modest and did not seem to differ in subjects without a myocardial infarction preceding HF onset. Our results suggest that there may be several genomic regions associated with HF in older adults, and support the hypothesis that common genetic variation, regardless of the clinical mechanism responsible for reduced cardiac output in HF, contributes to the risk. These findings merit replication in other community-based settings of incident HF.

Conclusions: We identified 2 loci that were associated with incident HF and exceeded genome-wide significance. The findings merit replication in other community-based settings of incident HF.

Relation of Platelet and Leukocyte Inflammatory Transcripts to Body Mass Index in the Framingham Heart Study

Summary: There have been many genetic epidemiology and biomarker studies examining associations of common genetic variants (DNA) and circulating proteins with clinically apparent cardiovascular disease and associated risk factors; however, there has been relatively little study of gene expression or transcriptomics. Quantitative differences in the abundance of transcripts (messenger RNA) has been demonstrated in specific malignancies, but gene expression from a large community-based cohort examining cardiovascular disease or its risk factors has never been reported. In this study, we measured quantitative expression of 48 genes in 1846 participants of the Framingham Offspring cohort from RNA derived from isolated platelets and leukocytes. Specific inflammatory platelet-derived transcripts were significantly associated with higher body mass index. Compared with platelets, fewer leukocyte-derived transcripts were associated with body mass index or other cardiovascular risk factors. Select transcripts were found to be highly heritable. This study demonstrates that inflammatory transcripts derived from platelets, particularly those part of inflammatory regulating pathways, are associated with BMI, whereas other distinct transcripts, many known to be related to platelet function, are heritable. This is the first study, using a large community-based cohort, to demonstrate that quantitative gene expression is associated with risk factors, most notably body mass index.

Conclusions: Inflammatory transcripts derived from platelets, particularly those part of the nuclear factor \(\kappa\) B pathway, are associated with BMI, whereas other are heritable. This is the first study, using a large community-based cohort, to demonstrate clinical correlates of gene expression and is consistent with the hypothesis that specific peripheral-blood transcripts play a role in the pathogenesis of coronary heart disease and its risk factors.

Genetic Regulation of Serum Phytosterol Levels and Risk of Coronary Artery Disease

Summary: Phytosterols interfere with intestinal cholesterol absorption; therefore, supplementation of phytosterols in “functional foods” (eg, margarines, yogurts) is widely used for their potential to lower serum cholesterol. A certain fraction (usually \(< 5\%\)) of phytosterols is absorbed. Even though the human body has very efficient means of eliminating these sterols, supplementation of phytosterols in functional foods has been shown to double their serum levels. Other than that, plasma sterol levels are under tight genetic control, with heritability estimates of \(< 80\%\). We performed a genome-wide association study investigating genetic variability of serum phytosterol levels in the general population. We found that \(< 10\%\) of the variability of phytosterol levels was explained by 3 independent genetic variants at the ATP-binding cassette hemitransporter \(ABCG8\) and \(ABO\) blood group gene loci. Because there is growing evidence for a potential proatherogenic role of phytosterols, we investigated whether genetic variants affecting phytosterols also modulate coronary artery disease risk. Strikingly, we found that common genetic variants associated with increased serum phytosterol levels increase coronary artery disease risk as well. Our findings might have potential relevance with regard to the use of phytosterols as food supplements.

Conclusion: Common variants in \(ABCG8\) and \(ABO\) are strongly associated with serum phytosterol levels and show concordant and previously unknown associations with CAD.

Genetic Determinants of Major Blood Lipids in Pakistanis Compared With Europeans

Summary: Levels of the major blood lipids, LDL-C, HDL-C, and triglyceride are each strongly associated with the risk of coronary heart disease (CHD). Several genetic variants have been established in the regulation of lipid metabolism in people of European continental ancestry; however there are few data available on the genetic determinants of these lipid traits in South Asians a population with a high burden of cardiometabolic conditions. We investigated 45 000 variants across 2000 genes in 3200 Pakistanis, and 2450 Germans using the same gene array. A total of 41 variants at 14 loci, were found to be significantly associated with major lipid traits in Pakistanis compared with Europeans. This study suggests that several lipid-related genetic variants are common to Pakistanis and Europeans, though they explain only a modest proportion of population variation in lipid concentration. Allelic frequencies and effect sizes of lipid-related variants can differ between Pakistanis and Europeans.

Conclusions: Several lipid-related genetic variants are common to Pakistanis and Europeans, though they explain only a modest proportion of population variation in lipid concentration. Allelic frequencies and effect sizes of lipid-related variants can differ between Pakistanis and Europeans.

Fine-Mapping in African Americans of 8 Recently Discovered Genetic Loci for Plasma Lipids: The Jackson Heart Study

Summary: A principal goal of genetic association studies has been to augment current disease prediction algorithms by identifying genetic variants associated with common diseases. Genome-wide association (GWA) studies have identified many novel loci associated with plasma lipid traits. However, most GWA studies published to date have been conducted exclusively in samples of European ancestry. Therefore, it is unclear whether these loci are relevant in a broader range of ethnic groups such as African Americans. Results from GWA studies direct interest to regions of association in the genome, but, typically, the causal variants remain unknown. Many have
postulated that densely genotyping these regions in African Americans may lead to identification of the causal variant because of the unique genetic architecture on the African ancestral background. In the current study, we tested polymorphisms from loci identified through GWA and fine-mapped regions in the Jackson Heart Study, a community-based cohort of African American individuals in Jackson, Miss. Our study addresses whether the same genetic loci identified in populations of European ancestry through GWA will be associated with blood lipids and, therefore, possibly predictive in African Americans. We also demonstrate the advantages and disadvantages of using a fine-mapping approach in African Americans to identify causal variants within the associated genomic regions.

Conclusions: We confirm that 5 genetic regions associated with lipid traits in European-derived populations are relevant in African Americans. To further evaluate these loci, fine-mapping in larger African American cohorts and/or resequencing will be required.

Association of Genetic Risk Variants With Expression of Proximal Genes Identifies Novel Susceptibility Genes for Cardiovascular Disease

Summary: Over recent years, variations in the DNA sequence, so-called single nucleotide polymorphisms (SNPs), have been increasingly acknowledged as genetic modulators of the risk of developing cardiovascular disease. Several large-scale genome-wide association studies have identified hundreds of SNPs associated with cardiovascular disease or important risk factors. It is usually assumed that the gene located closest to the SNP site is the so-called “risk gene” responsible for the effect of the SNP, but this may not be true. In this investigation, we sought to identify the culprit genes likely to mediate the effect of SNPs associated with cardiovascular disease. By combining gene expression measurements in liver, plaque and vessel wall with 156 SNPs associated to cardiovascular disease, we demonstrate that the effect of SNPs are tissue- and phenotype-specific. Furthermore, in more than 20 instances, the culprit gene was not directly deducible from the location of the risk-SNP. An important promise of genome-wide association studies is the ability to identify novel pathobiological pathways and new concepts for therapy. To fully understand the role of SNPs in mediating disease and to develop novel treatments, accurate knowledge of the functional consequences of genetic variation is essential. The present study emphasizes the necessity of combining functional studies of genetic markers with association data to realize the full potential of current genomic research.

Conclusions: This study demonstrates several instances of association between risk-SNPs and genes immediately adjacent to them. It also demonstrates instances in which the associated gene is not the immediately proximal and obvious candidate gene for disease. This shows the necessity of careful studies of genetic marker data as a first step toward application of genome-wide association studies findings in a clinical setting.

Dynamic microRNA Expression Programs During Cardiac Differentiation of Human Embryonic Stem Cells: Role for miR-499

Summary: The discovery and isolation of human embryonic stem cells (hESCs) have given medical science the tantalizing prospect of one day regenerating organs and tissues in human patients. This is because hESCs are pluripotent, which enables them to differentiate into virtually any cell type of the human body. However, forcing these cells to change their phenotype is an imperfect science and is often time-consuming, resource-intensive, and plagued by poor yields. Fundamentally, what is needed is better control over the factors that induce, maintain, and repress pluripotency and differentiation. MicroRNAs (miRNAs) are a newly discovered endogenous class of small RNAs that play important regulatory roles by targeting messenger RNAs for cleavage or translational repression. hESCs are known to express miRNAs that are often undetectable in adult organs, and a growing body of evidence has implicated miRNAs as important arbiters of heart development and disease. Here, we report the first miRNA profiling study of cardiomyocytes derived from hESCs and identify miRNAs, such as miR-499 and -1, that are strongly associated with cardiac differentiation. With the ultimate goal of discovering factors that might enhance cardiac differentiation, we then selected miR-499 and -1 for functional studies. Each miRNA had significant, though different, positive effects on cardiac differentiation, suggesting a powerful role for miRNAs in influencing hESC fate decisions. In the future, we believe miRNAs may play a key role in achieving higher yields of hESC-derived cardiomyocytes that can then be used for transplantation studies and, ultimately, clinical therapies.

Conclusions: Taken together, our data give significant insight into the regulatory networks that govern human embryonic stem cell differentiation and highlight the ability of miRNAs to perturb, and even control, the genes that are involved in cardiac specification of human embryonic stem cells.

Improved Prediction of Cardiovascular Disease Based on a Panel of Single Nucleotide Polymorphisms Identified Through Genome-Wide Association Studies

Summary: In the past 3 years, several genome-wide association studies have identified common genetic variants at multiple loci that are significantly associated with coronary artery disease (CAD) risk. The most robust of these is a risk allele at 9p21 that has been shown to modestly but inconsistently improve CAD risk prediction over and above conventional risk factors. However, the extent to which additional recently identified genetic variants of more modest effect size cumulatively improve risk prediction remains controversial. In the present study, we demonstrate, in 2 large CAD case-control populations, that a collective of 12 previously replicated CAD-associated single nucleotide polymorphisms confers significantly greater predictive capabilities than do traditional risk factors or traditional risk factors plus 9p21. We also show that logistic regression performs better than more complicated machine-learning approaches. Large meta-analyses of CAD case-control data sets including >100 000 individuals are underway. Relevant to the goal of personalized medicine, more informative risk prediction models can be expected as additional CAD-associated single nucleotide polymorphisms are identified and more refined phenotypic data become available.

Conclusions: Using the collective of 12 SNPs confers significantly greater predictive capabilities for CAD than 9p21, 3, whether traditional risks are or are not considered. More accurate models probably will evolve as additional CAD-associated SNPs are identified.

Design of the Coronary Artery Disease Genome-Wide Replication And Meta-Analysis (CARDIoGRAM) Study: A Genome-Wide Association Meta-Analysis Involving More Than 22 000 Cases and 60 000 Controls

Summary: Despite the recent progress in identification of coronary artery disease (CAD)/myocardial infarction genes, only a relatively limited fraction (<10%) of the overall genetic risk (heritability) of the disease is explained by the currently identified loci. One part of the explanation is likely to be the limited power of individual genome-wide association studies to detect such loci. The global Coronary Artery Disease Genome-Wide Replication And Meta-analysis (CARDIoGRAM) consortium will now analyze genome-wide information from >22 000 cases of CAD and >60 000 controls, and this will undoubtedly identify additional loci harboring common variants affecting CAD risk. Indeed, we anticipate a wealth
of new information on heritable aspects of CAD and its risk factors, which likely will open multiple opportunities for scientific exploration. However, such a large experiment requires careful prospective planning of the methodology used. Here we describe how such a meta-analysis, including a replication study, could be conducted. In conclusion, CARDIoGRAM is a novel and powerful consortium poised to contribute to the understanding of common genetic variation affecting the risk for CAD and myocardial infarction. This information then can be used to derive mechanistic information on biological processes as well as used to identify potential targets for therapeutic intervention.

Conclusion: CARDIoGRAM is poised to contribute to our understanding of the role of common genetic variation on risk for CAD and MI.7

Proteomic Analysis of Lung Tissues From Patients With Pulmonary Arterial Hypertension

Summary: Pulmonary arterial hypertension is characterized by marked vascular remodeling, evident microscopically as pronounced structural changes to pulmonary arteries and arterioles. This study analyzed and compared the abundance of >300 proteins in lungs from patients with end-stage pulmonary arterial hypertension and healthy donors. The levels of 25 proteins varied between the 2 groups. The majority of proteins that were increased in abundance were associated with cell growth, proliferation, and cell metabolism. Among the new findings was increased expression of chloride intracellular channel 4, a multifunctional protein involved in angiogenesis. It is a target for several of the signaling pathways implicated in pulmonary arterial hypertension—transforming growth factor-β, vascular endothelial growth factor, and bone morphogenetic protein—and on immunohistochemistry was found to localize predominantly to endothelial cells in occlusive and plexiform vascular lesions in the diseased lung. This raises the possibility of a role in the disorganized angiogenesis of plexiform lesions and as such a potential target for addressing directly the vascular pathology. This study demonstrates the potential of proteomics to provide an unbiased analysis of the protein changes underlying pulmonary arterial hypertension to better understand the disease to inform new treatments and biomarkers.

Conclusions: Label-free proteomics identified differences in the expression of several proteins in the pulmonary arterial hypertension lung, many of which are relevant to the disease process. Increased expression of chloride intracellular channel 4 may be pertinent to the disorganized angiogenesis of plexiform lesions.18

Multiple Genetic Loci Influence Serum Urate Levels and Their Relationship With Gout and Cardiovascular Disease Risk Factors

Summary: Although the role of serum urate in the causal pathway for gout has been well characterized, substantial controversy exists regarding whether elevated serum urate also may be a cause of high blood pressure (BP), hyperglycemia, and chronic kidney disease; whether the association with serum urate observed in observational studies merely is a consequence of these conditions; or whether it is an artifact of uncontrolled confounding factors. Because the association between genes and disease generally is not subject to confounding by environmental factors or reverse causality, causal inferences between exposure and disease can be examined more specifically using Mendelian randomization. In the present investigation, we tested the association of a genetic score constructed using 8 loci associated with serum urate with coronary heart disease (CHD) and its risk factors, including gout, glucose, systolic BP, diastolic BP, estimated glomerular filtration rate, and chronic kidney disease. Except for gout, none of the associations was statistically significant, and the lack of associations was replicated in another equally large independent sample. Although further confirmation is warranted, our study helps to elucidate the relationship between serum urate and CHD and its risk factors, which may contribute to a better understanding of the usefulness of controlling serum urate for preventing and designing treatments for CHD and its risk factors.

Conclusions: The genetic urate score analysis suggested a causal relationship between serum urate and gout but did not provide evidence for one between serum urate and cardiovascular risk factors and CHD.19

Association of Single-Nucleotide Polymorphisms From 17 Candidate Genes With Baseline Symptom-Limited Exercise Test Duration and Decrease in Duration Over 20 Years: The Coronary Artery Risk Development in Young Adults (CARDIA) Fitness Study

Summary: Cardiorespiratory fitness-related phenotypes have been shown to have a substantial genetic component in numerous epidemiological studies. However, identifying common genetic variants associated with changes in health-related fitness phenotypes over time in large population-based studies has proven difficult because of the lack of appropriate data sets and the feasibility of measuring fitness in a large group of people. We observed in the CARDIA Fitness Study that carrying several favorable alleles at loci shown to be independently associated with baseline exercise test duration or with change in exercise duration resulted in a >2.5-minute longer baseline treadmill time in blacks and a 1-minute lesser decrease in treadmill time over 20 years in whites. Thus, these data provide evidence of both the independent and the joint effects of multiple markers from several candidate genes on symptom-limited exercise test duration in young adults and with change in exercise test duration over 20 years. Symptom-limited exercise tests have important clinical relevance for evaluating exercise tolerance because many individuals may not be able to perform a true maximal exercise test to exhaustion. These results are of interest to clinicians because exercise duration in CARDIA participants was shown to be predictive of the development of cardiovascular disease risk factors such as diabetes, hypertension, and metabolic syndrome. Understanding the underlying genetic basis of the ability to benefit from regular exercise can likely facilitate custom-tailored programs and therapies for individuals with less-favorable genotypes.

Conclusions: In multimarker constructs, alleles at genes related to skeletal muscle Na+/K+ transport, hypoxia, and mitochondrial metabolism are associated with symptom-limited exercise test duration over time in adults.20

Gene Coexpression Network Topology of Cardiac Development, Hypertrophy, and Failure

Summary: High-throughput techniques allow insight into differential regulation of genes in diverse disease states. Investigation of higher-order interactions between genes in networks provides a rich context for exploring fundamental biological questions. Using advanced statistical methods for network analysis of the largest collection of myocardial gene expression data assembled to date, we tested a fundamental hypothesis in cardiovascular biology, namely, whether a fetal gene expression program is recapitulated in the failing and hypertrophied adult heart. In doing so, we identified networks of genes in developing, failing, and hypertrophied myocardium that may be important to both the developing and failing heart. Limited evidence was found for a single, coordinated program of gene expression shared between the developing and failing heart. However, the definition of higher-order organization of the myocardial transcriptome and novel potential transcriptional regulators enriches our understanding of the genomic basis of myocardial development and disease and may someday lead to therapeutic interventions targeting maladaptive cardiac stress responses.
Conclusions: Network modeling allows systems analysis of cardiovascular development and disease. Although we did not find evidence for a global coordinated program of fetal gene expression in adult myocardial adaptation, our analysis revealed specific gene expression modules active during both development and disease and specific candidates for their regulation.

Heterogeneity of the Phenotypic Definition of Coronary Artery Disease and Its Impact on Genetic Association Studies

Summary: The clinically heterogeneous nature of coronary artery disease (CAD) has caused well-recognized limitations in the phenotypic characterization of cases and controls for genetic studies of CAD. Experts in the field have recommended the use of standardized phenotypic definitions (eg, myocardial infarction and angiographic measures of disease burden) for all genetic analyses, although there currently is no empirical evidence to support the use of these “purist” phenotypes. In this project, we described the degree of heterogeneity of phenotypic definitions in individual genetic studies and investigated, with meta-analytic techniques, the impact of phenotypic definitions on the consistency and magnitude of genetic effects for CAD. We analyzed 965 individual studies for 32 genetic associations and found that the CAD phenotypes could be classified into 3 categories: acute coronary syndromes (44%); angiographically documented disease (34%); and broad, not otherwise specified CAD (22%). However, these clinical phenotypes were overlapping. Subgroup meta-analyses by phenotype showed discordant results, but phenotypic classification generally explained small proportions of between-study heterogeneity. No CAD phenotype was consistently associated with larger or more homogeneous genetic effects in meta-analyses to support its preferential use in genetic studies or meta-analyses for CAD. Our findings reinforce the need of considering the totality of evidence for CAD phenotypes in future meta-analyses of genetic studies. Phenotypic-specific effects at the clinical phenotype level may exist, but these should be explored in secondary analyses after an association between a genetic marker and CAD has been established by an all-inclusive meta-analysis.

Conclusions: Substantial phenotypic heterogeneity exists in CAD genetic associations, but differences in phenotype definition make a small contribution to between-study heterogeneity. We did not find a consistent effect in terms of the magnitude or homogeneity of summary effects for a specific phenotype to support its preferential use in genetic studies or meta-analyses for CAD.

Clinical Events as a Function of Proton Pump Inhibitor Use, Clopidogrel Use, and Cytochrome P450 2C19 Genotype in a Large Nationwide Cohort of Acute Myocardial Infarction: Results From the French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) Registry

Summary: Over the past months, there has been an intense scientific debate on the potential clinical impact of proton pump inhibitor use in patients treated with clopidogrel. There is a potential for drug-drug interactions because many proton pump inhibitors are metabolized by or are inhibitors of the cytochrome P450 2C19 (CYP2C19) enzyme. The stakes are considerable, given the huge number of patients treated with these medications. Specifically, the impact of CYP2C19 genetic polymorphisms on clinical outcomes in patients receiving a proton pump inhibitor and clopidogrel has not been evaluated. Overall, proton pump inhibitor use was not associated with an increased risk for any of the main outcomes (in-hospital and 1-year survival, 1-year myocardial infarction— and stroke-free survival, 1-year major ischemic events in hospital survival, in-hospital bleeding and transfusion) in either the overall population or any of the subgroups tested. One of the key new findings of the present analysis is that there was no clinically relevant association in adverse cardiovascular events or mortality among patients with no or 1 CYP2C19 loss-of-function allele. Thus, in this population, the results do not support the avoidance of proton pump inhibitor use for those patients receiving clopidogrel who are at increased risk of gastrointestinal bleeding. However, because of the low number of patients and resultant large confidence interval ranges, the possibility that a higher early risk may exist in patients with 2 CYP2C19 variant alleles cannot be dismissed and needs further clinical studies. We believe that these results are important for physicians in charge of these patients.

Conclusion: PPI use was not associated with an increased risk of cardiovascular events or mortality in patients administered clopidogrel for recent MI, whatever the CYP2C19 genotype, although harm could not be formally excluded in patients with 2 loss-of-function alleles.

Upregulation of the 5-Lipoxygenase Pathway in Human Aortic Valves Correlates With Severity of Stenosis and Leads to Leukotriene-Induced Effects on Valvular Myofibroblasts

Summary: Aortic valve stenosis is a complex potentially modifiable inflammatory process with a spectrum of disease ranging from aortic sclerosis to severe destroyed valvular architecture, akin to atherosclerosis, and has become the most common indication for surgical valve replacement. The diseased valve is characterized by pathological remodeling and pronounced calcification leading to obstruction of the left ventricle outflow tract. Factors predicting the transition from early potentially modifiable stages of the valvular disease to manifest stenosis have not yet been fully elucidated. Echocardiography is the key tool for assessment of stenosis severity, and clinical decision making is based on its results. The inflammatory environment within the affected aortic valve stimulates the 5-lipoxygenase pathway leading to production of potent inflammatory mediators, leukotrienes. In the present study, a unique macroscopic dissection technique was used to model in vivo disease development, representing the entire disease spectrum from early signs to more advanced morphological changes. In addition, correlation analyses between echocardiographic parameters quantifying the stenosis severity and the quantitative gene expression data obtained from the thickened part revealed significant influence of several downstream components of the leukotriene pathway on stenosis severity in an early potentially modifiable stage of the valve disease. The translational implication of our data are that pharmacological intervention using leukotriene receptor antagonists could potentially retard the hemodynamic progression.

Conclusions: The upregulation of the leukotriene pathway in human aortic valve stenosis and its correlation with clinical stenosis severity, taken together with the potentially detrimental leukotriene-induced effects on valvular myofibroblasts, suggests one possible role of inflammation in the development of aortic stenosis.

The S1103Y Cardiac Sodium Channel Variant Is Associated With Implantable Cardioverter-Defibrillator Events in Blacks With Heart Failure and Reduced Ejection Fraction

Summary: Sudden cardiac death (SCD) risk stratification for primary prevention implantable cardioverter-defibrillators (ICDs) is currently limited to reduced ejection fraction, yet the majority of people having SCD do not meet this criteria, nor do the majority of patients receiving primary prevention ICDs derive benefit from the device. In this report, we show that the S1103Y variant in the cardiac sodium channel gene, present in approximately 13% of blacks, is associated with a 3- to 4-fold increase in ICD “events” in patients with reduced ejection fraction. This genetic association is indepen-
dent of traditional clinical risk factors for SCD. Identifying patients with this variant may potentially improve risk stratification for SCD in blacks, in whom there is a high prevalence of this variant.

Conclusions: This is the first report that the S1103Y variant is associated with a higher incidence of ventricular arrhythmias in blacks with heart failure and reduced ejection fraction.25

Chromosome 9p21 Haplotypes and Prognosis in White and Black Patients With Coronary Artery Disease

Summary: To date, the chromosome 9p21 region is the best-replicated independent genetic risk factor for development of coronary artery disease (CAD), usually as incident myocardial infarction, among whites. However, whether this genetic marker is a predictor of adverse outcomes among patients with established CAD is unclear. Given that our ability to predict which individuals will develop adverse outcomes is limited, and direct-to-consumer genetics companies are offering genotyping of this locus to patients, an understanding of how well previously noted associations apply to different patient populations is important. Accordingly, we investigated whether alleles of 9p21 single-nucleotide polymorphisms (SNPs) previously associated with increased risk for incident CAD also were associated with increased risk for adverse cardiovascular outcomes among patients with previously documented CAD. We used data from 2 cohorts: 1 enrolled with chronic stable CAD (International Verapamil SR Trandolapril Study) and 1 enrolled at the time of an acute coronary syndrome (Investigation of Outcomes From Acute Coronary Syndromes Study). Both cohorts were followed for at least 2 years for cardiovascular outcomes. We found the 9p21 SNPs previously associated with higher risk for incident CAD/myocardial infarction were not associated with long-term outcomes among patients with established CAD. Several other studies also have noted no association between 9p21 and prognosis, and some suggest a protective effect associated with these SNPs among patients with established CAD. Caution, therefore, is warranted when interpreting genetic tests of the 9p21 locus because the risk associated with an allele may differ depending on the patient population.

Conclusions: Our findings suggest that previously reported chromosome 9p21 SNPs, which predict incident CAD, are not associated with higher risk for adverse outcomes in patients with established CAD. The less commonly reported linkage disequilibrium block warrants further investigation.26

Genetic Variations in the α2A-Adrenoreceptor Are Associated With Blood Pressure Response to the Agonist Dexmedetomidine

Summary: The α2A-adenoreceptor (α2A-AR) is a key regulator of central and peripheral sympathetic tone and thus cardiovascular responses. Alpha2A-AR agonists are in clinical use as anesthetic agents (dexmedetomidine) or antihypertensive agents (clonidine). Functional variants in the α2A-AR gene (ADRA2A) have previously been shown to affect receptor expression and function. Furthermore, in recent studies, some variants in or near ADRA2A were associated with platelet aggregation response to epinephrine, increased receptor expression, reduced insulin secretion, and increased risk for diabetes mellitus. However, the effects of ADRA2A variants on cardiovascular regulation and response to agonists in humans are unclear. In this single-masked, placebo-controlled, translational study in 73 healthy subjects, we infused increasing doses of the selective α2A-AR agonist dexmedetomidine under controlled conditions and measured the decrease in blood pressure, heart rate, and plasma catecholamine concentrations. We then genotyped 9 ADRA2A tagging variants, derived haplotypes, and analyzed the response to dexmedetomidine according to ADRA2A genotypes and haplotypes. There was substantial interindividual variability in blood pressure responses to dexmedetomidine. Common genetic ADRA2A variants and haplotypes previously linked to greater or lesser gene transcription/expression were associated with approximately 100% greater and 40% lesser responses to the drug, respectively. Thus, ADRA2A genotype explained some of the interindividual variability in drug response. Our findings may have implications for the prediction of blood pressure reduction (both as therapeutic and adverse effects) in response to α2A-AR agonists such as clonidine and dexmedetomidine. Further studies will be necessary to define the contribution of ADRA2A variants to drug response in the clinical setting.

Conclusions: Common ADRA2A variants are associated with the hypotensive response to dexmedetomidine. Effects of specific variants/haplotypes in vivo are compatible with their known effects on gene expression in vitro.27

Comparative Lipidomics Profiling of Human Atherosclerotic Plaques

Summary: Although lipids of human atherosclerotic plaques have been analyzed previously, target-focused measurements restricted to individual lipid classes remain insufficient to reveal the global lipid imbalances in atherosclerosis. We have adapted a liquid extraction-based surface sampling device for the analysis of plaque lipids from tissue sections. For quantitation, shotgun lipidomics was performed on lipid extracts using the different scan options of a triple quadrupole mass spectrometer. In total, 150 lipid species from 9 different classes were identified, of which 24 were detected in endarterectomies only. A comparison of 29 carotid endarterectomy specimens revealed lipid signatures of symptomatic-asymptomatic lesions as well as stable-unstable plaque areas. This comprehensive analysis of plaque lipids highlights the importance of measuring individual lipid species rather than lipid classes to obtain insights into the lipid heterogeneity within atherosclerotic lesions.

Conclusions: This comprehensive analysis of plaque lipids demonstrates the potential of lipidomics for unraveling the lipid heterogeneity within atherosclerotic lesions.28

Coronary Artery Endothelial Transcriptome In Vivo: Identification of Endoplasmic Reticulum Stress and Enhanced Reactive Oxygen Species by Gene Connectivity Network Analysis

Summary: Phenotype differences among arterial, venous, microvascular, and lymphatic vessels are well established during development. However, endothelial heterogeneity resulting from local environmental conditions also occurs between regions of the same vessel. In the arterial system the local physical, chemical, and fluid mechanical environment varies spatially to create regions where persistent changes in endothelial gene and protein expression promote atherogenesis. Blood flow characteristics, therefore, are determinants of endothelial phenotype plasticity and, by association, localized atherosusceptibility. Lesion locations map to arterial geometries that promote flow disturbances, typically near branches, bifurcations, and vessel curvatures. In this study, comparisons of endothelial gene expression between arteries as well as between sites within the same artery in a normal swine population were analyzed by a systems biology approach that constructed gene coexpression networks to detect differences in endothelial transcripts that may be related to regions of known susceptibility to atherosclerosis. The results reveal a sharp distinction between coronary and noncoronary arterial endothelial phenotypes while simultaneously identifying phenotypes associated with atherosusceptibility common to both categories. In atherosusceptible regions of both noncoronary and coronary arteries, there was significant enrichment of biological functions related to endoplasmic reticulum stress, an adaptive response that corrects excess unfolded protein synthesis in the cell and is linked to locally increased levels of reactive oxygen species. Of note, coronary artery endothelium showed both a more extensive...
Protein Kinase D2 Controls Cardiac Valve Formation in Zebrafish by Regulating Histone Deacetylase 5 Activity

Summary: Defective development of the heart valves occurs in 20% to 30% of congenital malformations. However, in most cases, the underlying causes have not been identified. There is increasing evidence that the regulatory mechanisms governing normal valve development also contribute to human valve pathology. In searching for novel molecular signaling pathways that orchestrate vertebrate heart valve development, we isolated the molecular cause of the ethynitrosourea (ENU)-induced recessive embryonic-lethal zebrafish mutant bungee (bng), which shows defective endocardial cushion development and subsequently impaired heart valve formation. We found that the bng mutation selectively impairs Protein kinase D2 kinase activity, which leads to reduced Histone deacetylase 5 phosphorylation, nuclear export, and inactivation. As a result of enhanced Histone deacetylase 5 repressor activity, Notch signaling is severely impaired in bungee mutant embryos. Accordingly, expression of the well-known Notch target genes Hey1, Hey2, and HeyL, is decreased in bng mutant embryos. Hence, it will be interesting to evaluate in future studies whether mutations in components of this novel signaling pathway such as Protein kinase D2, Histone deacetylase 5, Krüppel-like factor, Notch1, and members of the Hey family are also involved in human congenital heart disease, especially those that arise from defective endocardial cushion development such as septal and valvular defects. Furthermore, our findings might affect future strategies aiming to engineer human heart valve tissue for improved therapeutics or replacement strategies.

Conclusions: We demonstrate for the first time that proper heart valve formation critically depends on Protein kinase D2-Histone deacetylase 5-Krüppel-like factor signaling.

H11 Kinase/Heat Shock Protein 22 Deletion Impairs Both Nuclear and Mitochondrial Functions of STAT3 and Accelerates the Transition Into Heart Failure on Cardiac Overload

Summary: Cardiac overload represents a major cause of heart failure and triggers the activation of multiple stress-activated pathways, including the expression of the heat shock protein H11 kinase/Hsp22 (Hsp22). The objective of the present study was to determine the specific function of Hsp22 in a context of overload. This goal was achieved by generating a knockout mouse model of Hsp22 deletion. We show that, under pressure overload generated by aortic constriction, deletion of Hsp22 accelerates the transition into heart failure and increases mortality. Specifically, Hsp22 represents a necessary and previously undescribed activator of the stress-regulated transcription factor STAT3. We also illustrate that Hsp22 couples the activity of STAT3 as a transcription factor, together with its novel function as an activator of mitochondrial respiration. Therefore, Hsp22 represents a novel mode of cardiac stress response by combining the adaptation of gene expression and that of mitochondrial respiration.

Conclusions: This study found that Hsp22 represents a previously undescribed activator of both nuclear and mitochondrial functions of STAT3, and its deletion in the context of pressure overload in vivo accelerates the transition into heart failure and increases mortality.

Common Variants in CASQ2, GPD1L, and NOS1AP Are Significantly Associated With Risk of Sudden Death in Patients With Coronary Artery Disease

Summary: Sudden cardiac death remains a public health problem of significant magnitude, and the key to prevention is improvement in risk-stratification methodology. Recent studies have shown that there is evidence of a genetic component even among patients with coronary disease who have sudden cardiac death, the most common yet complex form of this condition. We used high through-put genetic analysis to evaluate the potential role of genes that are known to be causative in more rare, familial forms of sudden cardiac death, such as the long-QT and Brugada syndromes. The results indicate that common variations in the genes known to be involved in the rare syndromes are also associated with sudden cardiac death in the more common and complex coronary artery disease manifestation. These findings provide evidence for a unifying genetic link between rare and common forms of sudden cardiac death and are likely to inform the development of enhanced risk-stratification methodologies.

Conclusions: Common variations in or near CASQ2, GPD1L, and NOS1AP are associated with increased risk of sudden cardiac death in patients with coronary artery disease. These findings provide further evidence for overlap between the genetic architecture of rare and common forms of sudden cardiac death, and replication in additional populations is warranted.

Increased microRNA-1 and microRNA-133a Levels in Serum of Patients With Cardiovascular Disease Indicate Myocardial Damage

Summary: Recently, it was reported that levels of muscle-specific microRNA (miRNA or miR) increased in the plasma or serum of patients with acute myocardial infarction (MI). However, it is still poorly understood from where or under what conditions miRNAs are released into the blood stream. We first show that muscle-specific miR-1 and miR-133a increased in the serum of patients with acute coronary syndrome, and these microRNA levels were elevated in the early phase after the onset of acute MI, when there was no increase of serum cardiac Troponin T. The expression levels of these miRNAs were correlated with the serum cardiac Troponin T levels. We also indicated that the miR-133a levels increased in the serum of patients with not only acute MI but also unstable angina pectoris and Takotsubo cardiomyopathy. Next, we attempted to determine the tissue distribution of miR-133a in a mouse model of MI and revealed that not only the infarcted region but also the border zone is the source of circulating miR-133a. Furthermore, in vitro experiments indicated that stimulation of calcium ionophore increased miR-133a release from cardiac myoblasts only at concentrations in which cell death was observed and the released miRNA was functional. Taken together, our data suggest that circulating miR-133a, which is derived from injured myocardium, can be used as a sensitive, early diagnostic biomarker for myocardial damage. Additionally, because released microRNAs can regulate gene expression in other cells, the present study may provide a new insight into the function of miRNA in the pathophysiology of MI.

Conclusions: These results suggest that elevated levels of circulating miR-133a in patients with cardiovascular diseases originate mainly from the injured myocardium. Circulating miR-133a can be used as a marker for cardiomyocyte death, and it may have functions in cardiovascular diseases.
A Genome-Wide Association Study Identifies LIPA as a Susceptibility Gene for Coronary Artery Disease

Summary: In relation to polygenic coronary artery disease, recent genome-wide association studies have revealed interesting novel loci whose pathophysiological significance is incompletely understood at present. Variation in gene expression may be an important intermediate link between common genetic variants and phenotypes. In our study, combining information from genome-wide association studies and global gene expression in peripheral blood monocytes, we identified interesting single-nucleotide polymorphisms in the LIPA (lysosomal acid lipid A) gene on chromosome 10q23 in relation to coronary artery disease. LIPA gene expression was also associated with endothelial function, an intermediate phenotype of coronary artery disease. Consistent associations at the genetic, gene expression, subclinical disease, and disease levels support a causal relationship and add a pathophysiological plausible candidate for future investigation in cardiovascular risk assessment as well as a potential therapeutic target at all stages of the disease process. The approach of combining genome-wide association studies information and global gene expression shows a successful way to further exploit genome-wide data in relation to coronary artery disease. If further confirmed, biomarkers of the lysosomal acid lipid A pathway may be candidate markers for risk prediction in primary and secondary prevention and may potentially serve as targets for interventional strategies if proven to be causal.

Conclusions: The use of data on genetic variants and the addition of data on global monocyteic gene expression led to the identification of the novel functional coronary artery disease (CAD) susceptibility locus LIPA, located on chromosome 10q23.31. The respective eSNPs associated with CAD strongly affect LIPA gene expression level, which was related to endothelial dysfunction, a precursor of CAD.

Reciprocal Transcriptional Regulation of Metabolic and Signaling Pathways Correlates With Disease Severity in Heart Failure

Summary: Congestive heart failure (HF) is a leading cause of morbidity and mortality worldwide. Despite phenotypic similarities characterized by ventricular dilatation and reduced contractility, the extent of common and divergent gene expression between different forms of HF remains a matter of intense debate. Focusing on molecular pathways, we demonstrate that myocardial gene expression in 28 experimental models of nonischemic (pressure overload, loading, chronic isoproterenol infusion, Chagas disease, and transgenic HF models) and ischemic HF models is consistently characterized by downregulation of major metabolic pathways and concomitant upregulation of cell signaling pathways, thus recapitulating a fetal gene expression program. In contrast to this uniform transcriptional response observed in animal models, human HF microarray studies displayed a greater variability, with some studies even showing a reversed pattern. The reasons for this divergence are almost certainly due to the atherosclerotic process, we identified interesting novel loci whose pathophysiological significance is incompletely understood at present. Variation in gene expression may be an important intermediate link between common genetic variants and phenotypes. In our study, combining information from genome-wide association studies and global gene expression in peripheral blood monocytes, we identified interesting single-nucleotide polymorphisms in the LIPA (lysosomal acid lipid A) gene on chromosome 10q23 in relation to coronary artery disease. LIPA gene expression was also associated with endothelial function, an intermediate phenotype of coronary artery disease. Consistent associations at the genetic, gene expression, subclinical disease, and disease levels support a causal relationship and add a pathophysiological plausible candidate for future investigation in cardiovascular risk assessment as well as a potential therapeutic target at all stages of the disease process. The approach of combining genome-wide association studies information and global gene expression shows a successful way to further exploit genome-wide data in relation to coronary artery disease. If further confirmed, biomarkers of the lysosomal acid lipid A pathway may be candidate markers for risk prediction in primary and secondary prevention and may potentially serve as targets for interventional strategies if proven to be causal.

Conclusions: The use of data on genetic variants and the addition of data on global monocyteic gene expression led to the identification of the novel functional coronary artery disease (CAD) susceptibility locus LIPA, located on chromosome 10q23.31. The respective eSNPs associated with CAD strongly affect LIPA gene expression level, which was related to endothelial dysfunction, a precursor of CAD.

Distinct Epigenomic Features in End-Stage Failing Human Hearts

Summary: Molecular research in heart failure points to the hypothesis that a generic cardiac genomic response is activated in the failing myocardium regardless of the original inciting cause. This cardiac genomic stress response is typified by the re-expression of fetal genes, upregulation of fibrosis-related genes, and others. It may also reflect unifying features of a failed myocardium such as fibrosis, altered energy use, vascular rarefaction, cell death, and altered contractility, all of which are features found in the failing myocardium again regardless of the inciting cause. The epigenome refers to “marks” on the genome, including DNA methylation and histone modification. The epigenome regulates the expression of underlying genes and, recent evidence suggests that part of the epigenome may be altered by diet and the environment. In our research, we have examined the genome-wide landscape of the epigenome of healthy and end-stage cardiomyopathic human hearts. We have determined that distinct epigenomic patterns exist in important DNA elements of the human cardiac genome in the failing myocardium. Individually, sites of differential epigenomic patterns may control the expression of specific genes with critical functions in the progression of heart failure. However whether the altered epigenome is simply a consequence in end-stage disease or actually contributes to disease progression remains to be determined. This work now opens an important new avenue of research because the epigenome may represent a drug discovery target for novel heart failure therapy.

Conclusions: Distinct epigenomic patterns exist in important DNA elements of the cardiac genome in human end-stage cardiomyopathy. The epigenome may control the expression of local or distal genes with critical functions in myocardial stress response. If epigenomic patterns track with disease progression, assays for the epigenome may be useful for assessing prognosis in heart failure. Further studies are needed to determine whether and how the epigenome contributes to the development of cardiomyopathy.

Homozygosity Mapping and Exome Sequencing Reveal GATAD1 Mutation in Autosomal Recessive Dilated Cardiomyopathy

Summary: Recognition of idiopathic dilated cardiomyopathy (DCM) as a heritable disorder in 25% to 50% of patients with this condition has provided a rationale for screening echocardiography at at-risk relatives to detect presymptomatic disease. Furthermore, familial DCM has been the impetus for human genetics investigations to uncover the molecular underpinnings of myopathic heart failure. DCM is now well established as a genetically heterogeneous disorder, yet mutations in identified genes are estimated to account for only one third of cases. Beyond targeted hypothesis-based screening of candidate genes, completion of the Human Genome Project and technological advances in DNA analysis have enabled powerful whole-genome approaches that overcome inherent limitations of traditional disease gene discovery. These approaches can, in fact, uncover unanticipated DCM genes and new insights into the pathobiology of heart failure. In this study, we identified and phenotypically characterized a consanguineous family with nonsyndromic DCM segregating as an autosomal recessive trait. Exome sequencing, providing comprehensive sequence data for protein-coding regions of virtually all genes, and genome-wide locus mapping, providing a positional filter for the large number of identified genetic variants, were used as synergistic strategies to
identify a pathogenic homozygous mutation in GATA1. Previous work has shown that GATA1 binds to histones, the fundamental building blocks of chromatin that package and condense DNA and serve as targets for posttranslational modification and regulation of gene expression. Consistent with murine DCM caused by genetic disruption of histone deacetylases, the data implicate an inherited basis for epigenetic perturbation as an underlying basis for human heart failure.

Conclusions: Linkage analysis and exome sequencing were used as synergistic genomic strategies to identify GATA1 as a gene for autosomal recessive dilated cardiomyopathy. GATA1 binds to a histone modification site that regulates gene expression. Consistent with murine DCM caused by genetic disruption of histone deacetylases, the data implicate an inherited basis for epigenetic dysregulation in human heart failure.

A Gene Expression Signature That Classifies Human Atherosclerotic Plaque by Relative Inflammation Status

Summary: Treatment of atherosclerosis would benefit from the improvements in diagnostic techniques and therapeutic treatments that could result from a greater understanding of the biology of atherosclerotic plaque. Toward this end, we have carried out a large scale analysis of gene expression patterns in atherosclerotic plaques from patients with peripheral artery disease. By analyzing 101 peripheral plaques, we identified 2 distinct sets of genes that tend to be expressed in a mutually exclusive manner in plaque. One set consists of genes whose expression is generally associated with activated T cells and macrophages; the other set consists of genes whose expression is associated with smooth muscle cells. On the basis of a large amount of previously reported histological analysis of atherosclerotic plaque, we believe these gene expression patterns are associated with relatively inflamed or stable plaque types, and we hypothesize that the inflamed pattern of gene expression may be associated with vulnerable plaque prone to thrombosis. In addition, it is possible that within the gene expression profiles we collected from these plaques, there will be indications that could suggest either novel diagnostic markers of plaque progression or stabilization or novel therapeutic targets.

Conclusions: The robust mRNA expression signature identified in the present report is associated with pathological features of vulnerable atherosclerotic plaque and may be useful as a source of biomarkers and targets of novel antiatherosclerotic therapies.

Genomic Risk Variants at 1p13.3, 1q41, and 3q22.3 Are Associated With Subsequent Cardiovascular Outcomes in Healthy Controls and in Established Coronary Artery Disease

Summary: The genomic risk loci at 1p13.3, 1q41, and 3q22.3 have been strongly associated with the risk of developing coronary heart disease in many large genome-wide association and cohort studies. This study investigated whether these important coronary artery disease risk variants may also contribute to disease progression and poorer outcomes in patients with established cardiovascular disease. Whether these risk loci also increase the risk of subsequently experiencing a cardiovascular event in individuals with no known overt cardiovascular disease at the time of recruitment also was assessed. The findings from this study support previous associations between 1p13.3 and myocardial infarction and lipid levels. Extending these findings, we observed in patients with coronary heart disease an association between 1p13.3 and readmission for non-ST-segment elevation MI and between 1q41 and the composite end point of death/cardiovascular disease readmission. The coronary artery disease risk locus at 3q22.3 was associated with subsequent survival/admission for cardiovascular disease event in individuals who were free of overt coronary artery disease at the time of study inclusion. These findings provide further evidence for an important role of 1p13.3, 1q41, and 3q22.3 in coronary artery disease. Furthermore, this study suggests that variants at 1p13.3 and 1q41 are independently associated with clinical outcomes in patients with established coronary heart disease and confirms 3q22.3 as a predictor of cardiovascular risk in individuals free of overt heart disease.

Conclusions: These data suggest that coronary artery disease genomic risk variants at 1p13.3 and 1q41 are associated with subsequent clinical outcome in heart patients and confirm rs9818870 at 3q22.3 as a predictor of cardiovascular risk in individuals free of overt heart disease.

Post-Genomic Update on a Classical Candidate Gene for Coronary Artery Disease: ESR1

Summary: After age, male sex is the most important risk factor for cardiovascular disease; however, little research has been carried out to understand the underlying causes of sex-related risk, in comparison with work on modifiable risk factors. Although the physiological differences between the sexes are evident, the genetic differences are minimal because sex is determined by the presence (in males) of a single gene, SRY, on chromosome Y. This leads to the important conclusion that male sex itself is not a cardiovascular risk factor but a proxy variable that captures a large fraction of risk via other unknown, possibly modifiable, metabolic factors that differ between males and females. Sex hormone metabolism is a prime candidate system for explaining sex differences in risk. In this highly powered study, the authors perform an extensive survey of a broad range of genetic variation in the ESR1 gene, which encodes the principal candidate for explaining sex-related differences in coronary artery disease (CAD) risk, Estrogen Receptor α, ERα. Despite ERα’s central role in sex hormone signaling, its widespread expression in vascular tissues, and the importance of sex for CAD risk, the authors find no evidence for the involvement of genetic variation in ESR1 in modifying CAD risk, either in the general population or separately in males and females. Against the context of a history of inconsistent results regarding this question, this study provides a reasonably conclusive answer and may stimulate a renewed effort to explore other elements of the sex hormone system to explain sex differences in CAD risk.

Conclusions: We suggest that future research on the genetic basis of sex-related differences in CAD risk should initially prioritize other genes in the reproductive steroid hormone biosynthesis system.

Effect of CYP2C19*2 and *3 Loss-of-Function Alleles on Platelet Reactivity and Adverse Clinical Events in East Asian Acute Myocardial Infarction Survivors Treated With Clopidogrel and Aspirin

Summary: CYP2C19*2 allele carriage has shown significant association with the antiplatelet response and ischemic event occurrence in patients who suffered acute coronary syndrome or who were treated with percutaneous coronary intervention during dual antiplatelet therapy with aspirin and clopidogrel. Asian population has a high prevalence of the CYP2C19 loss-of-function (LOF) genotype compared with white population (~70% versus ~35%), with the CYP2C19*3 LOF allele and considerable portion of poor metabolizers (10~15%). In Asian survivors of acute myocardial infarction, platelet reactivity increases proportionally according to the number of the CYP2C19 LOF allele (*2 or *3), which is related to a high prevalence of the consensus-defined high on-treatment platelet reactivity (more than 50%). The CYP2C19 LOF allele (*2 or *3) carriage is an important predictor of ischemic events, but long-term clinical outcome seems similar or lower compared with whites. The influence of the CYP2C19*3 allele on clopidogrel response and clinical outcome is as strong as the CYP2C19*2 allele.
Conclusions: Among East Asian patients who survived an acute myocardial infarction, the CYP2C19 LOF allele carriage appears to affect clopidogrel pharmacodynamics and cardiovascular events according to the number of the CYP2C19 LOF allele; the influence of the CYP2C19*2 and *3 alleles on clopidogrel response and long-term outcomes does not differ.\(^1\)

**Genome-Wide Association Study for Coronary Artery Calcification With Follow-Up in Myocardial Infarction**

**Summary:** Coronary artery calcification, detected by computed tomography, is a noninvasive measure of coronary atherosclerosis and an independent predictor of myocardial infarction. We conducted a genome-wide association study with a discovery sample of nearly 10,000 participants of European origin and a replication sample of >6000 participants of European origin. We report strong evidence for genetic variants in 9p21 near the CDKN2B and CDKN2A genes and in 6p24 within the PHACTR1 gene. Additionally, we found evidence for concordance of single-nucleotide polymorphism associations with both coronary artery calcification and some but not all other loci known to be associated with myocardial infarction, including 3q22 (MRAS gene), 13q34 (COL1A1/COL1A2 genes), and 1p13 (SORT1 gene). These findings reinforce the important role of variation at multiple genes in the pathogenesis of coronary atherosclerosis. Although the functional mechanism of many of these genes is not fully understood, our comprehensive study lays the groundwork for future studies to determine the role of several genes in treatment, prediction, and prevention of coronary atherosclerosis and resulting myocardial infarction and other clinically apparent forms of coronary heart disease.

**Conclusions:** SNPs in the 9p21 and PHACTR1 gene loci were strongly associated with coronary artery calcification (CAC) and myocardial infarction (MI), and there are suggestive associations with both CAC and MI of SNPs in additional loci. Multiple genetic loci are associated with development of both underlying coronary atherosclerosis and clinical events.\(^2\)

**Excess of Rare Variants in Non-Genome-Wide Association Study Candidate Genes in Patients With Hypertriglyceridemia**

**Summary:** Polygenic hypertriglyceridemia (HTG) is a complex disease trait defined by fasting age- and sex-adjusted plasma triglyceride (TG) concentration >95th percentile. Our emerging understanding of the genetic architecture of HTG is that common small-effect and rare large-effect variants in several genes that modulate plasma TG metabolism act together to increase susceptibility to HTG. However, despite recent progress, much of the genetic susceptibility to HTG remains unattributed. Modern genomic technologies including genome-wide association studies have implicated many genes in disease susceptibility, although they seem to have largely exhausted their potential for continued gene discovery. Accordingly, alternative hypotheses and experimental designs are required to explain the unattributed variation among HTG patients. We tested the hypothesis that candidate genes identified by mouse models and human monogenic phenotypes of TG metabolism, including APOC2, CREB3L3, GPHB1, LMF1, and ZBH3, would harbor an excess of rare large-effect variants in HTG patients versus control subjects. In total, we identified 47 rare variants in 413 HTG patients and 16 rare variants in 324 healthy control subjects, corresponding to a 2.3-fold accumulation in HTG patients versus control subjects \((P<0.0050)\). Rare variant accumulation was most pronounced in APOC2, CREB3L3, and LMF1, suggesting that rare loss-of-function mutations in these genes contribute significantly to HTG predisposition in their carriers. Therefore, this study underscores the utility of alternative candidate gene selection criteria, and the power of emerging analytic strategies including rare variant accumulation, to implicate novel genes in disease susceptibility.

**Conclusions:** These extensive resequencing studies show a significant accumulation of rare genetic variants in non-GWAS candidate genes among patients with polygenic hypertriglyceridemia, and indicate the importance of testing specific hypotheses in large-scale resequencing studies.\(^3\)

**Common Genetic Variation in the 3'-BCL11B Gene Desert Is Associated With Carotid-Femoral Pulse Wave Velocity and Excess Cardiovascular Disease Risk: The AortaGen Consortium**

**Summary:** Carotid-femoral pulse wave velocity (CFPWV) is a heritable measure of aortic stiffness that is strongly associated with increased risk for major cardiovascular disease events. However, the molecular mechanisms contributing to aortic stiffness remain largely undefined. To evaluate associations of common genetic variants with CFPWV, we conducted a meta-analysis of genome-wide association data in 9 community-based European ancestry cohorts consisting of 20,634 participants. Results were replicated in 2 additional European ancestry cohorts involving 5306 participants. We identified a highly significant locus of association at 14q32.2 in the VRK1-BCL11B gene desert in a linkage disequilibrium block that harbors 1 or more gene enhancers. We also showed that variation at this locus is associated with increased risk for major cardiovascular disease events, providing strong support for the hypothesis that increased CFPWV contributes to the pathogenesis of cardiovascular disease. We demonstrated that 2 ncRNAs as well as flanking genes, BCL11B and VRK1, are expressed in human aorta. Further work will be required to define precise mechanisms mediating the association between CFPWV and genetic variation in the VRK1-BCL11B gene desert. Elucidation of pathways affected by this locus will provide new insights into the process of aortic stiffening in humans and could yield potential targets for specific interventions that reverse or attenuate aortic stiffening and prevent the associated morbidity and mortality.

**Conclusions:** Common genetic variation in a locus in the BCL11B gene desert that is thought to harbor 1 or more gene enhancers is associated with higher CFPWV and increased risk for cardiovascular disease. Elucidation of the role this novel locus plays in aortic stiffness may facilitate development of therapeutic interventions that limit aortic stiffening and related cardiovascular disease events.\(^4\)

**References**


Metabolomics, Proteomics, Genomics, and Transcriptomics in Circulation and the Circulation Subspecialty Journals
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