Connexin Gene Transfer Preserves Conduction Velocity and Prevents Atrial Fibrillation

Atrial fibrillation is the most common arrhythmia found in clinical practice, affecting 2 to 5 million in the US and several million more worldwide. The presence of atrial fibrillation substantially increases individual risk of stroke, heart failure, and death. A principle limitation to clinical practice is the lack of safe effective therapies for this pervasive arrhythmia. We previously reported a gene-painting method capable of 100% transmural gene transfer to all parts of the atria accessible from an open-chest pericardium approach. In the current report, we used this method to transduce the atria with either connexin 40 or 43, the 2 principle atrial gap junction proteins. We found that connexin gene transfer had no measurable effect on sinus rhythm animals but that gene transfer with either connexin preserved atria conduction velocity and prevented atrial fibrillation. These data suggest that gap junctions are not the principle limitation to conduction velocity under normal circumstances but that impaired gap junction conductance is a factor in atrial fibrillation. We saw no evidence of proarrhythmia or other safety concerns. The painting method should be directly applicable to the problem of postoperative atrial fibrillation. With modifications to increase duration of gene expression and to reduce the invasive nature of delivery, the method should be applicable to general atrial fibrillation. Formal preclinical testing is required before clinical investigation. See p 216.

Aortic Event Rate in the Marfan Population: A Cohort Study

Marfan syndrome is a genetic disease usually related to a mutation in the gene coding for FBN1. It is transmitted as a dominant autosomal disease. The genetic nature of the disease allows early diagnosis with familial screening. Progressive aortic dilatation leading to aortic dissection and rupture is the main life-threatening complication associated with the syndrome. Medical management includes β-blocker therapy and prohibition of intensive sports. Regular follow-up visits are crucial to evaluate the aortic diameter with echocardiography and the need for prophylactic aortic surgery. In the present study, the management of 732 patients followed these rules, and the value of 50 mm was our threshold for preventive aortic surgery. Using this strategy, we showed that the risk of aortic event (dissection, rupture, or sudden death) was $<0.05$/y in the population with an aortic diameter $<50$ mm in the absence of known risk factors such as pregnancy, familial history of aortic dissection at a low diameter, or rapid increase in diameter. This risk increases with the diameter of the aorta at the level of the sinuses of Valsalva; this increase was $>4$-fold in the patients whose aorta was 50 to 54 mm compared with 45 to 49 mm. The 50-mm threshold at the level of Valsalva appears to be a reasonable threshold for proposing preventive aortic surgery in patients with Marfan syndrome. See p 226.

Evaluation of Multiple Biomarkers of Cardiovascular Stress for Risk Prediction and Guiding Medical Therapy in Patients With Stable Coronary Disease

The benefit of angiotensin-converting enzyme inhibitors in low-risk patients with stable coronary artery disease without heart failure remains controversial, and current practice guidelines note that it is reasonable but not recommended to use angiotensin-converting enzyme inhibitors when cardiovascular risk factors are well-controlled and revascularization has been performed. We now demonstrate that elevated levels of 3 novel biomarkers of cardiovascular stress, midregional pro-atrial natriuretic peptide, midregional pro-adrenomedullin, and C-terminal pro-endothelin-1, are associated with the subsequent risk of cardiovascular death and heart failure independent of clinical factors (adjusted hazard ratios per 1-SD increase of 1.97, 1.48, and 1.47, respectively: $P=0.002$ for each biomarker). Furthermore, elevated levels of these biomarkers identified patients in whom therapy with an angiotensin-converting enzyme inhibitor resulted in a significant reduction in the risk of cardiovascular death or heart failure. Specifically, trandolapril significantly reduced the risk of cardiovascular death or heart failure in patients who had elevated levels of $\geq 2$ biomarkers (hazard ratio, 0.53; 95% confidence interval, 0.36–0.80), whereas there was no benefit in patients with elevated levels of 0 or 1 biomarker (hazard ratio, 1.09; 95% confidence interval, 0.74–1.59; $P_{\text{interaction}}=0.012$). Thus, in patients with stable coronary artery disease and preserved left ventricular ejection fraction, elevated levels of novel biomarkers of cardiovascular stress identify patients who are at higher risk of cardiovascular death and heart failure and may be useful to select patients who derive significant benefit from angiotensin-converting enzyme inhibitor therapy. See p 233.

Associations Between Lipoprotein(a) Levels and Cardiovascular Outcomes in Black and White Subjects: The Atherosclerosis Risk in Communities (ARIC) Study

On the basis of observational and prospective studies with limited statistical power, lipoprotein(a) [Lp(a)] is not considered a risk factor for cardiovascular disease in blacks. The Adult Treatment Panel III guidelines also note that “although Lp(a) levels are higher in African Americans than in Caucasians, an increased risk for coronary heart disease associated with higher Lp(a) levels in African Americans has not been documented.” This is despite the fact that Lp(a) levels in blacks are 2 to 4 times higher than those in whites. In these analyses, we evaluated the association between plasma Lp(a) levels and the risk of incident cardiovascular disease events (incident coronary heart disease and incident ischemic strokes) in 3467 black and 9851 white participants in the Atherosclerosis Risk in Communities (ARIC) study over a 20-year follow-up period. Our analyses show that Lp(a) levels were positively associated with cardiovascular disease events in both races. Associations were at least as strong in blacks, with a larger range of relevant Lp(a) concentrations in blacks than in whites. Elevated Lp(a) levels should therefore be considered to be associated with an increased cardiovascular disease risk in blacks. See p 241.

Comprehensive Use of Cardiopulmonary Exercise Testing Identifies Adults With Congenital Heart Disease at Increased Mortality Risk in the Medium Term

Cardiopulmonary exercise testing (CPX) has emerged as an important tool for risk stratification in adults with congenital heart disease. However, it remains uncertain how best to apply CPX parameters in clinical practice. Relating a CPX result to prognosis may be difficult for clinicians because previous studies based on conventional statistical analyses provide relative risk estimates rather than actual projected risk. Moreover, it is unclear how CPX parameters should be combined to best obtain prognostic information. We assessed the relation between CPX parameters and midterm survival in 1375 adult...
congestive heart disease patients at a tertiary center over a 10-year period (median follow-up, 5.8 years). Estimated 5-year survival rates as a function of peak oxygen consumption, heart rate reserve, and ventilation per unit of carbon dioxide production (Ve/VC\textsubscript{O2} slope) were calculated. The combination of peak oxygen consumption and heart rate reserve provided the greatest predictive information after adjustment for clinical parameters such as negative chronotropic agents, age, and presence of cyanosis. However, the incremental value of these exercise parameters was reduced in patients with peak respiratory exchange ratio <1.0. CPX provides strong prognostic information in adult patients with congenital heart disease. Prognostication should be approached differently, depending on the presence of cyanosis, use of rate-lowering medications, and achieved level of exercise. We provide 5-year survival prospects based on CPX parameters in this growing population. See p 250.

**Using Stress Testing to Guide Primary Prevention of Coronary Heart Disease Among Intermediate-Risk Patients: A Cost-Effectiveness Analysis**

In an era of low-cost statin therapy, universal statin therapy for patients at intermediate risk of coronary heart disease may be more cost effective than using noninvasive testing to select which patients should receive statins and aspirin. Our analyses show that, under a wide variety of assumptions, asymptomatic intermediate-risk adults should not be screened with stress electrocardiography, stress ECG, or stress nuclear scanning to make decisions on statin treatment (in men and women) and aspirin treatment (in men). Rather, all such intermediate-risk patients should be treated. However, if adherence with universal statin treatment is <21% and if a positive stress test would raise adherence to 75%, stress ECG would save costs because of the increased adherence. Alternatively, if stress ECG testing were reserved for individuals who are nonadherent to statin and aspirin therapy, such testing would be cost effective if it raised adherence to \(\geq 5\)% and cost saving if it raised adherence to 13% in those who were previously nonadherent. The potential virtue of this approach to increasing adherence requires further study and comparison with alternative interventions to increase adherence. Regardless, routine statin (men and women) and aspirin (men) treatment is warranted without prior stress testing in all intermediate-risk men and women. See p 260.

**Determinants and Consequences of Renal Function Variations With Aldosterone Blocker Therapy in Heart Failure Patients After Myocardial Infarction: Insights From the Eplerenone Post–Acute Myocardial Infarction Heart Failure Efficacy and Survival Study**

In patients with left ventricular systolic dysfunction with or at high risk of heart failure in the postinfarction setting, adding eplerenone to standard care improves outcome even if these patients exhibit moderate reductions in estimated glomerular filtration rate, despite causing a slightly greater early reduction in estimated glomerular filtration rate compared with placebo. An early decline in estimated glomerular filtration rate by >20% was associated with worse cardiovascular outcomes independently of baseline estimated glomerular filtration rate and of the use of eplerenone, which nevertheless retained its prognostic benefits even under such circumstances. Eplerenone did not alter the usual long-term decline in renal function that occurs in this elderly population. See p 271.

**Serial Measurement of Cardiac Troponin T Using a Highly Sensitive Assay in Patients With Chronic Heart Failure: Data From 2 Large Randomized Clinical Trials**

Validation of biomarkers requires large cohorts reflecting a broad range of contemporary patients. In 5284 patients with chronic heart failure from 2 independent randomized clinical trials, the Valsartan Heart Failure Trial (Val-HeFT) and the Gruppo Italiano per lo Studio della Sopravvivenza nell’Insufficienza Cardiaca–Heart Failure (GISSI-HF), changes over time in circulating high-sensitivity cardiac troponin T were related to fatal and nonfatal outcomes. In both studies, increases in high-sensitivity cardiac troponin T levels over 3 to 4 months were associated with age, diabetes mellitus, and reduction in estimated glomerular filtration rate. Despite very low baseline levels, increases in high-sensitivity cardiac troponin T concentration were strongly associated with clinical outcome, even after adjustment for conventional risk factors and benchmark circulating biomarkers of cardiac function (N-terminal pro-brain natriuretic peptide) or inflammation (C-reactive protein). The discriminative value of changes in high-sensitivity cardiac troponin T was limited, however. The strength of these observations lies in the fact that the data were obtained in 2 independent samples that differed with respect to the origin of patients, length of follow-up, and prescription rate of comediations (with \(\beta\)-blockers and aldosterone receptor antagonists more frequent in GISSI-HF). These results are consistent with reports of worsening outcomes with increasing circulating troponin concentrations in community-dwelling older adults, in ambulatory patients with chronic heart failure, or in patients with chest pain. Although such changes may reflect normal biological variation, their relation with future adverse events regardless of baseline concentrations suggests that they may truly reflect dynamic changes in disease progression. Given the limited variability of troponin levels over time, the potential for using their changes to guide therapy of chronic heart failure is questionable. See p 280.

**Pulmonary Capillary Wedge Pressure Augments Right Ventricular Pulsatile Loading**

Right ventricular dysfunction is a major independent predictor of death in patients with elevated afterload from pulmonary hypertension. We analyzed data from >1000 right heart catheterizations to show that, unlike the systemic circulation, pulmonary vascular resistance (R\textsubscript{PVR}) and compliance (C\textsubscript{PVR}) display a highly predictable, inverse correlation and that this relationship is not significantly altered by pulmonary hypertension or severe pulmonary fibrosis and is altered only minimally by aging. This means that, unlike the proximal aorta in the systemic circulation, the main pulmonary artery contributes relatively little to overall C\textsubscript{PVR} and is little affected by age. The consistent R\textsubscript{PVR}-C\textsubscript{PVR} relation allows the prediction of R\textsubscript{PVR} decline required by a given treatment to adequately lower C\textsubscript{PVR} and thus reduce pulsatile and net right ventricular afterload. Current monotherapies for pulmonary hypertension are not reducing pulsatile afterload in most patients, identifying an area of clinical need. We also found that the R\textsubscript{PVR}-C\textsubscript{PVR} relationship is sensitive to changes in pulmonary venous pressure (mean pulmonary capillary wedge pressure) because elevation of this pressure lowers C\textsubscript{PVR} for any given R\textsubscript{PVR}. Augmenting right ventricular pulsatile afterload in patients with exertional dyspnea and a preserved ejection fraction who exhibit a marked rise in pulmonary capillary wedge pressure during exercise, we found a disproportionate decline in C\textsubscript{PVR} and thus increased pulsatile load. This identifies a novel mechanism whereby left-side diastolic dysfunction contributes to right ventricular load. See p 289.
An Unsuspected Property of Natriuretic Peptides: Promotion of Calcium-Dependent Catecholamine Release Via Protein Kinase G-Mediated Phosphodiesterase Type 3 Inhibition

Natriuretic peptides have long been viewed as compensatory hormones that are upregulated in the setting of heart failure, affording beneficial cardiac and hemodynamic effects. Yet a recent large randomized trial with recombinant B-type natriuretic peptide (nesiritide) failed to reduce mortality or rehospitalization in heart failure patients. We tested whether unsuspected proadrenergic effects might oppose the anticipated benefits of natriuretic peptides. We report that brain natriuretic peptide increases norepinephrine release in the guinea pig heart ex vivo, an effect that is further enhanced in ischemia/reperfusion. In addition, natriuretic peptides elicit catecholamine exocytosis in sympathetic nerve terminals isolated from the guinea pig heart and in nerve growth factor–differentiated rat pheochromocytoma PC12 cells, a model of sympathetic nerve endings. This proexocytotic effect is likely due to a protein kinase G-mediated inhibition of phosphodiesterase type 3. This increases intraneuronal cyclic AMP levels and protein kinase A activity, which culminates in increased intracellular calcium and norepinephrine release. Notably, these effects occur at concentrations of natriuretic peptides reached at cardiac sympathetic nerve endings in advanced congestive heart failure. We propose that this proadrenergic action may counteract any beneficial cardiac and hemodynamic effects of increasing natriuretic peptide levels in congestive heart failure and thus explain the ineffectiveness of nesiritide as a cardiac failure medication. See p 298.

Rates of Cardiac Catheterization Cancelation for ST-Segment Elevation Myocardial Infarction After Activation by Emergency Medical Services or Emergency Physicians: Results From the North Carolina Catheterization Laboratory Activation Registry

Regional ST-segment elevation myocardial infarction (STEMI) systems of care continue to develop and evolve, and now many metropolitan areas and states are organizing their efforts to provide timely reperfusion and intervention for an increasing number of patients. These systems now incorporate emergency medical services agencies and emergency departments as key drivers of their programs. It is important to acknowledge that activation of STEMI systems of care will inherently result in some degree of overtriage or false-positive activations if attempts are made to maximize the sensitivity in identifying all STEMI cases. In this statewide registry, including 14 percutaneous coronary intervention–capable hospitals receiving STEMI patients from their emergency medical services providers and referral hospitals, >3000 patients were followed to determine whether STEMI system activation was deemed to be appropriate or inappropriate, and the ultimate manner of treatment was recorded. The odds of having an appropriate system activation varied by means of hospital presentation and institution type where the activation occurred, with the greatest odds of having an appropriate STEMI system activation occurring at percutaneous coronary intervention–capable hospitals. By better understanding these issues, particular system components such as ECG interpretation and catheterization laboratory candidacy issues can be identified and can serve as a focus of continued process improvement and education. See p 308.

Notch Activation of Jagged1 Contributes to the Assembly of the Arterial Wall

Notch signaling is an evolutionarily conserved pathway that has been implicated in several forms of congenital heart disease and vascular pathologies. Aalamile syndrome, a multisystem disorder associated with mutations in the Notch2 receptor or the Notch ligand Jagged1, results in both cardiac defects such as pulmonary artery stenosis and tetralogy of Fallot and a variety of vascular lesions. These vascular lesions may be the result of defective smooth muscle development. The vascular smooth muscle of the aortic arch arteries provides for the eventual smooth muscle of much of the mature cardiac outflow tract and is derived from neural crest cells. In this article, we demonstrate a critical role for an autoamplification of Notch signaling in cardiac neural crest during vascular smooth muscle development. Notch activation, mediated by endothelial Jagged1, stimulates smooth muscle differentiation of undifferentiated mesenchyme to become vascular smooth muscle. This smooth muscle differentiation is accompanied by an upregulation of Jagged1 expression that activates Notch in subsequent neural crest cells. This lateral induction is mediated by a newly identified enhancer within the Jagged1 genomic locus, demonstrating that Jagged1 is a direct transcriptional target of Notch in vascular smooth muscle. Interruption of this signaling loop, through inhibition of Notch signaling or deletion of the Jagged1 receptor in the neural crest, results in reproducible forms of congenital heart disease involving the outflow tract of the heart. The elucidation of Jagged1 and Notch regulation during smooth muscle development will aid our understanding of congenital abnormalities and vascular defects associated with Jagged1/Notch deficiencies. See p 314.

A Randomized, Double-Blind, Placebo-Controlled, Dose-Ranging Study of Oral Sildenafil Citrate in Treatment-Naive Children With Pulmonary Arterial Hypertension

Pulmonary arterial hypertension is an important cause of morbidity and mortality in children and adults. Currently, 8 drugs are approved for adult pulmonary arterial hypertension, yet no drugs are approved for children. Because of similar clinical characteristics and histopathology, treatment for children has been extrapolated from evidence-based adult guidelines. However, better information is required to provide optimal pediatric dosing and to ensure safety in children of all ages. The 16-week, randomized, double-blind, placebo-controlled Sildenafil in Treatment-Naive Children, Aged 1 to 17 Years, With Pulmonary Arterial Hypertension (STARTS-1) study evaluated the effects of sildenafil in childhood pulmonary arterial hypertension. Treatment-naïve children with pulmonary arterial hypertension (n=234; aged 1–17 years; >8 kg) received low-, medium-, or high-dose sildenafil or placebo orally 3 times daily. Peak oxygen consumption, measured only in children who were able to reliably perform exercise testing (using cycle ergometry), was the primary end point. Hemodynamic parameters and World Health Organization functional class were assessed across all patients, including those unable to reliably perform exercise testing. Although the primary comparison of percent change in peak oxygen consumption for the 3 sildenafil groups combined was only marginally statistically significant, the improvements in exercise capacity, functional class, and hemodynamics with medium- and high-dose sildenafil suggest efficacy with these doses. Combined with interim data from the ongoing extension study, the overall profile favors the medium dose. Further investigation is warranted to determine optimal dosing based on age and body weight. See p 324.

Migraine Mutations Increase Stroke Vulnerability by Facilitating Ischemic Depolarizations

Our study establishes a mechanism that links migraine and stroke, 2 highly prevalent and debilitating diseases. Migraine is a well-recognized stroke risk factor. Although its prevalence is on par with
other stroke risk factors such as diabetes mellitus and hypertension, there has been little insight into the mechanism of the migraine-stroke association. Here, we present compelling evidence indicating that glutamatergic hyperexcitability associated with migraine mutations renders the brain more susceptible to ischemic depolarizations. As a result, the minimum critical level of blood flow required for tissue survival (ie, viability threshold) is elevated and infarction ensues, even in mildly ischemic tissues. This represents a paradigm shift in the search for a mechanism for increased stroke risk in migraineurs and differs radically from those previously postulated on the basis of clinical data alone. Our conclusions are based on optical and magnetic resonance imaging and electrophysiological recordings in transgenic mouse models for familial hemiplegic migraine type 1, a monogenic migraine syndrome (mutations in Ca,2.1 channels) that has been a model for common but genetically complex forms of migraine based on shared clinical features, glutamatergic mechanisms, and elevated stroke risk. Clinical implications include a shorter therapeutic window for acute stroke interventions in migraineurs because of accelerated loss of potentially salvageable penumbra. Furthermore, migraine prophylaxis may reduce stroke risk by suppressing cerebral hyperexcitability, and antithrombotic prophylaxis may be indicated in susceptible migraineurs because they are more likely to have infarcts if and when they develop cerebral ischemic events. See p 335.

**Clinical Implications of Electrocardiographic Left Ventricular Strain and Hypertrophy in Asymptomatic Patients With Aortic Stenosis: The Simvastatin and Ezetimibe in Aortic Stenosis Study**

This is the first study to examine the predictive value of ECG left ventricular strain and hypertrophy during watchful waiting in asymptomatic patients with aortic stenosis. In analyses of 1533 patients with asymptomatic mild to moderate aortic stenosis (aortic peak flow velocity ≥2.5 and ≤4.0 m/s) included in the Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) study, cardiovascular event rates were considerably higher in those with ECG left ventricular strain or hypertrophy. Despite similar aortic stenosis severity (aortic peak flow velocity ≥3.0 m/s), annual risks of heart failure were 1.4% and 0.4% in those with and without ECG strain, respectively. The presence of ECG left ventricular strain and hypertrophy remained significantly associated with poor prognosis also when adjusted by aortic valve area index and mean aortic gradient or when the analyses were updated with annual reexaminations. Thus, low-cost and easily accessible ECG left ventricular strain and hypertrophy data provide valuable tools for risk stratification in patients with aortic stenosis. Whether ECG strain identifies those whose prognosis would be improved by earlier aortic valve replacement merits further study. Finally, treatment with low-dose simvastatin does not influence the progression of ECG left ventricular hypertrophy or strain. See p 346.

**A, Adenosine Receptor Regulates Hyperlipidemia and Atherosclerosis**

The Western high-fat diet has long been associated with obesity, elevation in circulating cholesterol and triglycerides, and altered metabolic disorders. The major problem or the ultimate outcome is some form of cardiac occlusion or atherosclerosis. Although numerous studies have shown the negative effect of high-fat, high-cholesterol diets on the vasculature, including atherosclerosis and consequent mortality from occlusions of cardiac vessels, the general population would not change eating patterns to account for it. This has called for consideration of treatments that can potentially be used without a change in diet. Here, we used a genetically modified mouse model lacking the A, adenosine receptor and a specific agonist for this receptor, BAY 60-6583, to identify a new link between receptor expression/activation, lipidemia, and atherosclerosis. Our study showed augmented cholesterol and triglyceride levels and atherosclerosis in the knockout mice and a significant reduction in these parameters on treatment of wild-type mice with BAY 60-6583. Of importance, we observed a reduction in atherosclerosis across the aortic tree with this treatment while the mice were on a high-fat diet. We propose that activation of the A, adenosine receptor can be a therapeutic target that can reduce cholesterol and triglyceride levels and the progression of atherosclerosis without a significant change in the intake of a Western diet. See p 354.

**Extramedullary Hematopoiesis Generates Ly-6Chigh Monocytes That Infiltrate Atherosclerotic Lesions**

Atherosclerosis is an inflammatory disease characterized by the accumulation of lipids and leukocytes in the arterial wall. Monocytes are large circulating leukocytes believed to be essential to the development and exacerbation of atherosclerosis. As disease worsens, the number of circulating monocytes rises, whereas in models with monocyte depletion, atherosclerosis neither develops nor evolves. It is believed that hematopoietic progenitors give rise to circulating monocytes exclusively in the bone marrow. These medullary monocytes circulate, accumulate in tissue, and differentiate to macrophages or dendritic cells. Extramedullary sites such as the spleen maintain reservoirs of undifferentiated monocytes that can exit en masse in response to acute inflammation. In this study, we show that during atherosclerosis the bone marrow outsources the production of monocytes to the spleen. These extramedullary monocytes accumulate in the growing atheroma. From a clinical perspective, this finding is important because it identifies the spleen as a possible biomarker organ and therapeutic target for cardiovascular disease, and it proposes that inflammatory hematopoiesis could be targeted therapeutically in atherosclerosis. See p 364.