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Systems Genomics Identifies a Key Role for Hypocretin/Orexin Receptor-2 in Human Heart Failure

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ABSTRACT

BACKGROUND The genetic determinants of heart failure (HF) and response to medical therapy remain unknown. We hypothesized that identifying genetic variants of HF that associate with response to medical therapy would elucidate the genetic basis of cardiac function.

OBJECTIVES This study sought to identify genetic variations associated with response to HF therapy.

METHODS This study compared extremes of response to medical therapy in 866 HF patients using a genome-wide approach that informed the systems-based design of a customized single nucleotide variant array. The effect of genotype on gene expression was measured using allele-specific luciferase reporter assays. Candidate gene transcription-deficient mice underwent echocardiography and treadmill exercise. The ability of the target gene agonist to rescue mice from chemically-induced HF was assessed with echocardiography.

RESULTS Of 866 HF patients, 136 had an ejection fraction improvement of 20% attributed to resynchronization (n = 83), revascularization (n = 7), tachycardia resolution (n = 2), alcohol cessation (n = 1), or medications (n = 43). Those with the minor allele for rs7767652, upstream of hypocretin (orexin) receptor-2 (*HCRTR2*), were less likely to have improved left ventricular function (odds ratio: 0.40 per minor allele; $p = 3.29 \times 10^{-5}$). In a replication cohort of 798 patients, those with a minor allele for rs7767652 had a lower prevalence of ejection fraction >35% (odds ratio: 0.769 per minor allele; p = 0.021). In an HF model, *HCRTR2*-deficient mice exhibited poorer cardiac function, worse treadmill exercise capacity, and greater myocardial scarring. Orexin, an HCRTR2 agonist, rescued function in this HF mouse model.

CONCLUSIONS A systems approach identified a novel genetic contribution to human HF and a promising therapeutic agent efficacious in an HF model. (J Am Coll Cardiol 2015;66:2522–33) © 2015 by the American College of Cardiology Foundation.

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Heart failure (HF), a syndrome characterized by impaired function and high filling pressures, affects more than 5 million people in the United States and is expected to touch 3.5% of the population over the next 20 years (1). A wide range of conditions can lead to HF, such as coronary artery disease and hypertension (2). Although heritable (3), few studies have explored the genetic basis for HF. Candidate gene studies identified associations between common variants in *HSPB7* and *FRMD4B* and dilated cardiomyopathy (4) or advanced HF (5). Targeted genotyping of common variants in *ADRB1* and *GRK5*, members of the β-adrenergic receptor signaling pathway, demonstrated associations with survival (6,7). In the limited genome-wide studies of HF (8–11), only 1 common variant associated with dilated cardiomyopathy of genome-wide significance has been replicated (11).

Systems approaches to studying the genetics of complex traits have been successful in uncovering promising gene targets and identifying fundamental disease patterns. Genome-wide association analyses have been combined with gene expression and metabolic data, for example, to identify *AGPAT5* as an important effector of insulin resistance (12). Using gene coexpression network analyses, we previously identified patterns of gene expression found in common between diseased and developing myocardium (13). Gene expression patterns can also distinguish patients with HF and ischemic heart disease from those with dilated cardiomyopathy (14).

A phenomenon long recognized by HF physicians is that some patients dramatically respond to HF therapy with large increases in ejection fraction (EF) associated with positive remodeling of the left ventricle, whereas others deteriorate seemingly in spite of optimal medical management. Few studies have addressed even the clinical associations of such responders, a group for whom long-term survival is predictably better (15).

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In this study, our primary hypothesis was that there are genomic variants associated with a dramatic response to HF medical therapy. We used a systems approach to design a genetic discovery platform optimized for HF, then evaluated whether there was a functional role for the gene target of the variant association.

METHODS

STUDY POPULATIONS. Patients were recruited from Stanford University Medical Center, Stanford, California, and the Palo Alto Veterans Hospital, Palo Alto, California; patients were included who had clinically diagnosed HF, were referred for subspecialty care between 2005 and 2009, had an echocardiogram performed, and had an EF <55%. Patients were excluded if they could not be contacted by telephone or if they had insufficient clinical data. Also excluded were patients with congenital heart disease or cardiomyopathy due to infiltrative disease, a peripartum state, infection, or chemotherapy; with a myocardial biopsy suggestive of viral cardiomyopathy who responded to medical therapy within 30 days of administration; who experienced an acute myocardial infarction and showed subsequent improvement in their EF within 3 months of infarction; or with a history of substance abuse (illicit drugs or alcohol) within 6 months before the study. Patients whose EF improved after surgical or percutaneous revascularization, resynchronization therapy using biventricular pacing, alcohol cessation, or cardioversion were excluded from the genomic analyses. The EF was measured using routine transthoracic echocardiography obtained by trained echocardiographers using 2-dimensional scanning in the parasternal long axis, parasternal short axis, and apical views. Change in EF was measured as maximal difference between lowest recorded EF and the highest subsequent recorded EF. Written informed consent was obtained from study participants in accordance with the Stanford University Internal Review Board policy.

For the informative genome-wide association studies (GWAS), case subjects (n = 29) were patients whose EF had improved by >20% while on medical therapy. Patients with poor sample quality were excluded. Control patients (n = 37) were selected from those followed in the Stanford University transplant clinic who had demonstrated lack of clinical improvement before transplant despite medical therapy. The case and control patients were matched by age, sex, race, medical therapy received, baseline EF on echocardiography, type of cardiomyopathy, and duration of HF. The remaining HF patients underwent genotyping with the custom genotyping array. Patient charts were retrospectively reviewed and their baseline demographics, clinical characteristics, and medication use were recorded. Serial measurements from clinically available echocardiograms were also recorded. Outcome data were obtained through detailed phone interviews.

REPLICATION. For the independent replication study, patients with HF were recruited from the University of Pennsylvania as previously described (5). Briefly, Caucasian patients (n = 798) were

ABBREVIATIONS AND ACRONYMS

GWAS = genome-wide association study

HCRTR2 = hypocretin receptor-2

HF = heart failure

SNP = single nucleotide polymorphism

SNV = single nucleotide variant