A common variant in the β_2-adrenergic receptor and risk of sudden cardiac death

Michael C. Gavin, MD,* Christopher Newton-Cheh, MD, MPH,[†#] John Michael Gaziano, MD, MPH,[‡‖¶] Nancy R. Cook, ScD,[¶‡‡] Martin VanDenburgh,[¶] Christine M. Albert, MD, MPH[§¶**]

*From the *Cardiovascular Division, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, [†]Broad Institute of Harvard and MIT, Cambridge, Massachusetts, [‡]Massachusetts Veterans Epidemiology, Research and Information Center and Geriatric Research Education and Clinical Center, Boston VA Healthcare System, Boston, Massachusetts, [§]Center for Arrhythmia Prevention, [‖]Division of Aging, [¶]Division of Preventive Medicine, **Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, [#]Center for Human Genetic Research and Cardiovascular Research Center, Massachusetts General Hospital, Boston, Massachusetts, and [‡‡]Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts.*

BACKGROUND Homozygosity for a common nonsynonymous single nucleotide polymorphism (Gln27Glu) in the β_2-adrenergic receptor gene (*ADRB2*) has been inconsistently associated with sudden cardiac death (SCD) in individual studies of small sample size.

OBJECTIVE The purpose of this study was to examine the association between the Gln27Glu polymorphism and SCD in a large combined sample of SCD cases.

METHODS Nested case-control analysis was performed for individuals of Caucasian ancestry enrolled in six prospective cohort studies. Genotypes for the Gln27Glu variant were determined for 492 cases of SCD and 1,388 controls matched for age, sex, cohort, follow-up time, and history of cardiovascular disease (CVD) and at the time of the blood draw. Individual studies were combined with conditional logistic regression with fixed effects meta-analysis assuming a recessive model.

RESULTS Homozygosity for the Gln27 allele conferred a nonsignificant elevation of the age-adjusted odds ratio (OR 1.22, 95% confidence interval [CI] 0.98–1.53, $P = .08$) for SCD, which became marginally significant after controlling for multiple cardiac risk factors (OR 1.30, 95% CI 1.01–1.67, $P = .046$). In secondary analyses using controls additionally matched for the development of nonfatal CVD after the blood draw, results were attenuated (OR 1.19, 95% CI 0.92–1.52, $P = .19$). When the results of the primary analysis were combined in meta-analysis with published reports, a significant association between *ADRB2* genotype and SCD emerged (OR 1.35, 95% CI 1.15–1.60, $P = .0003$).

CONCLUSION These data from a large prospective case-control series, when combined with published studies, provide further evidence for an association between *ADRB2* genotype and SCD. The mechanism is unknown but appears to be partly mediated by development of CVD.

KEYWORDS Beta$_2$-adrenergic receptor; Genetic epidemiology; Sudden cardiac death; Sympathetic nervous system

ABBREVIATIONS ***ADRB2*** = β_2-adrenergic receptor gene; **β1AR** = β_1-adrenergic receptor; **β2AR** = β_2-adrenergic receptor; **CABS** = Cardiac Arrest Blood Study; **CAD** = coronary artery disease; **CHD** = coronary heart disease; **CHS** = Cardiovascular Health Study; **CI** = confidence interval; **CVD** = cardiovascular disease; **HERS** = Heart and Estrogen/Progestin Replacement Study; **HPFS** = Health Professionals Follow-up Study; **NHS** = Nurses' Health Study; **OR** = odds ratio; **PHS I** = Physicians' Health Study I; **PHS II** = Physicians' Health Study II; **SCD** = sudden cardiac death; **SNP** = single nucleotide polymorphism; **UCSF** = UCSF SCD Case Control Study; **WACS** = Women's Antioxidant Cardiovascular Study; **WHS** = Women's Health Study

(Heart Rhythm 2011;8:704–710)

Introduction

An estimated 250,000 to 400,000 sudden cardiac deaths (SCD) occur annually in the United States,[1,2] accounting for greater than 50% of all coronary heart disease (CHD).[3,4] The majority of these deaths occur as the first manifestation of heart disease[4,5]; therefore, reductions in SCD incidence will require improved risk stratification and preventive strategies within the general population. Because there is a heritable component to SCD risk within populations,[6–8] genetic markers may allow us to better identify individuals within the general population who are at an elevated risk for SCD when used in combination with other risk markers. In addition, improved understanding of the genetic determi-

Supported by Grant HL-68070 from the National Heart, Lung, and Blood Institute and a Lerner Young Investigator Award to Dr. Albert and a Doris Duke Charitable Foundation Clinical Scientist Development Award to Dr. Newton-Cheh. The cohort studies were supported by Grants HL-26490, HL-34595, HL-34594, HL-35464, HL-43851, HL-46959, and HL-80467 from the National Heart, Lung, and Blood Institute and Grants CA-34944, CA 40360, CA-47988, CA55075, CA-87969, and CA 97193 from the National Cancer Institute. **Address reprint requests and correspondence:** Dr. Christine M. Albert, Center for Arrhythmia Prevention, Division of Preventive Medicine, Cardiovascular Division, Brigham and Women's Hospital, 900 Commonwealth Avenue East, Boston, Massachusetts 02115-1204. E-mail address: calbert@partners.org. (Received September 13, 2010; accepted January 2, 2011.)

1547-5271/$ -see front matter doi:10.1016/j.hrthm.2011.01.003

nants of SCD within populations may illuminate biologic pathways involved in the genesis of lethal ventricular arrhythmias, which ultimately could lead to new therapeutic approaches for SCD prevention.

Neurohormonal activation through the adrenergic nervous system is recognized as an important pathway modulating vulnerability to ventricular arrhythmias in diverse disease states.[9,10] One of the first common genetic variants reported to be associated with SCD risk in population-based studies is a nonsynonymous single nucleotide polymorphism (SNP) resulting in an amino acid substitution (Gln27Glu, rs1042714) in *ADRB2* gene, which encodes the β_2-adrenergic receptors (β2AR), an important mediator of the cardiovascular response to sympathetic activation. Sotoodehnia et al[11] found an increased risk of SCD among an unselected population of elderly individuals homozygous for the Gln27 amino acid polymorphism and validated this finding in a younger population without overt heart disease. Subsequently, Tseng et al[12] failed to validate this association in two studies involving individuals with known coronary artery disease (CAD). The discrepant results could be due to small sample sizes of individual studies resulting in either spurious associations or lack of power to detect true associations. Alternatively, associations may differ with variable control for cardiovascular disease.

We sought to explore and attempt to replicate the previously reported association between the Gln27Glu polymorphism and SCD in a large combined sample of SCD cases among individuals of European ancestry assembled from six National Institutes of Health (NIH)-funded prospective cohorts using a prospective nested case-control design. We then combined these results with those previously reported among individuals of similar ethnicity in an expanded meta-analysis.

Methods

Study populations

Subjects in this nested case-control study were sampled from six prospective cohorts and clinical trials comprising a total of 40,878 men and 67,093 women with stored blood samples. The cohorts included the Physicians' Health Study I and II (PHS I and II), the Nurses' Health Study (NHS), the Health Professionals Follow-up Study (HPFS), the Women's Health Study (WHS), and the Women's Antioxidant Cardiovascular Study (WACS). The PHS I, WHS, and WACS studies initially were randomized trials of vitamin supplementation and/or aspirin. Treatment in these trials has ended, but prospective follow-up is ongoing in PHS I and WHS. PHS II is an ongoing randomized trial of vitamin supplementation. NHS and HPFS are observational cohort investigations. Information regarding medical history, incident disease, and lifestyle changes are assessed either annually or biennially by self-administered questionnaires.

Endpoint confirmation

The primary endpoint included cases of sudden and/or arrhythmic cardiac death that occurred after return of the blood sample and before April 1, 2007. A total of 540 sudden and/or arrhythmic deaths occurred among participants who donated blood samples at baseline, and 536 of these had DNA samples that passed our quality control standards. Because only 20 cases of SCD occurred among non-European ethnicities, analyses were limited to SCD cases among individuals of European ancestry.

Methods to document the timing and mechanism of cardiovascular deaths were similar across cohorts and have been described previously.[13] In brief, definite SCDs were defined as death or cardiac arrest occurring within 1 hour of symptom onset or those with an autopsy consistent with SCD (n = 412 [76.9%]). Unwitnessed deaths or deaths occurring during sleep were classified as probable SCDs if the subject was observed to be symptom-free in the preceding 24 hours and the circumstances of the death suggested that it could have been sudden (n = 92 [17.2%]). Deaths also were classified as arrhythmic or nonarrhythmic based on the definition of Hinkle and Thaler.[14] Arrhythmic death was defined as an abrupt spontaneous disappearance of pulse without evidence of prior circulatory impairment or neurologic dysfunction. Deaths that fulfilled the criteria for arrhythmic death but were preceded by more than 1 hour of symptoms (n = 32 [6.0%]) were also included in the combined endpoint of sudden and/or arrhythmic cardiac death. Among the total 536 sudden and/or arrhythmic deaths, autopsies were performed in 68 (12.7%) cases.

Selection of controls

For each case, up to three control subjects from the same risk set who were alive at the time of the SCD of the case were selected from the same cohort. Each case was matched for sex, age ($\pm$1 year), ethnicity, smoking status (current, never, past), time and date of blood sampling, fasting status, and presence or absence of cardiovascular disease (CVD), which included a history of myocardial infarction, angina, coronary artery bypass grafting, or stroke at the time of blood draw. Subjects within these cohorts are followed for CVD events on either an annual (WHS, WACS, PHS I and II) or biannual basis (HPFS, NHS). In an additional 69 cases, CVD developed after the blood draw but prior to SCD. For these cases, we selected a second set of three controls who also developed CVD between the time of the blood draw and the SCD of the case to explore how much of the overall association with SCD might be explained by development of nonfatal CVD prior to death.

Genotyping and quality control

Genomic DNA was extracted from the buffy coat fraction of centrifuged blood using Qiagen Autopure kits (Valencia, CA, USA) in NHS, HPFS, and WACS and from whole blood in PHS I. In WHS and PHS I, DNA was extracted using the MagNA Pure LC instrument using the MagNA Pure LC DNA isolation kit (Roche Applied Science, Penzberg, Germany). All assays were conducted without knowledge of case status, and samples were labeled by study code only. Matched case-control pairs were handled identically,