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Cumulative effects of common genetic variants on risk of sudden cardiac death

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ABSTRACT

Background: Genome-wide association studies and candidate-gene based approaches have identified multiple common variants associated with increased risk of sudden cardiac death (SCD). However, the independent contribution of these individual loci to disease risk is modest.
Objective: To investigate the cumulative effects of genetic variants previously associated with SCD risk.
Methods: A total of 966 SCD cases from the Oregon-Sudden Unexpected Death Study and 1926 coronary artery disease controls from the Wellcome Trust Case–Control Consortium were investigated. We generated genetic risk scores (GRSs) for each trait composed of variants previously associated with SCD or with abnormalities in specific electrocardiographic traits such as QRS duration, QTc interval and heart rate. GRSs were calculated using a weighted approach based on the number of risk alleles weighted by the beta coefficients derived from the original studies. We also compared the highest and lowest quintiles for the GRS composed of SCD SNPs.
Results: Increased cumulative risk was observed for a GRS composed of 14 SCD-SNPs (OR = 1.17 [1.05–1.29], P = 0.002). The risk for SCD was 1.5 fold greater in the highest risk quintile when compared to the lowest risk quintile (OR = 1.46 [1.11–1.92]). We did not observe significant associations with SCD for SNPs that determine electrocardiographic traits.
Conclusions: A modest but significant effect on SCD risk was identified for a GRS composed of 14 previously associated SCD SNPs. While next generation sequencing methodology will continue to identify additional novel variants, these findings represent proof of concept for the additive effects of gene variants on SCD risk.

1. Introduction

Sudden cardiac death (SCD) is a major public health problem responsible for more than 300,000 deaths in the United States on an annual basis [1–3]. Implantable defibrillators are effective in treating malignant tachyarrhythmias but their impact in primary prevention of SCD could be significantly enhanced by improvements in risk stratification [4–7]. Early studies highlighting a genetic contribution to pathophysiology of SCD [8–11] prompted multiple candidate-gene based studies and genome wide association studies (GWAS) [12,13]. As a result, several new loci associated with increased risk of SCD were identified from candidate gene based studies [14–20]. From GWAS studies, DNA variants within the BAZ2B [21] and CXADR [22] loci have passed the stringent GWAS significance threshold. However, independently, most of these loci contribute modestly to disease risk. In this study, we selected and investigated the contribution of common variants associated with SCD by published GWAS and candidate gene approaches. We also evaluated variants associated with intermediate electrical phenotypes (endophenotypes), specifically QRS duration [23], heart rate [24] and QTc interval [25]. We hypothesized that a genetic risk score (GRS) comprised of genetic variants that had been previously associated with SCD or SCD electrical

Abbreviations: CAD, coronary artery disease; GWAS, genome-wide association studies; GRS, genetic risk score; Oregon-SUDS, Oregon-Sudden Unexpected Death Study; SCD, sudden cardiac death; WTCCC, Wellcome Trust Case–Control Consortium.

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phenotypes would provide additional information on SCD risk prediction. To test this, we developed GRSs for each of these endophenotypes and tested their association with SCD, using a well characterized SCD sample from the Oregon Sudden Unexpected Death Study (Oregon-SUDS). As controls, we used subjects with coronary artery disease (CAD) from the Wellcome Trust Case Control Consortium Study (WTCCC), thus looking for variants that influence risk of SCD in individuals with CAD, constituting the most common form of SCD.

2. Methods

2.1. Ethics statement

This study was approved by the Institutional Review Boards of the Oregon Health and Science University, Cedars-Sinai Medical Center and all participant hospitals. The WTCCC Data Access Committee approved the use of the WTCCC data. All samples have been analyzed in accordance with the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all enrolled subjects.

2.2. Subjects

2.2.1. Oregon-SUDS

A total of 966 SCD cases of European descent from the Oregon-SUDS [26–28] with GWAS data available, were included in the study. The Oregon-SUDS is an ongoing prospective study of out-of-hospital SCD. Methods have been published in detail [26,28]. Briefly, SCD cases were identified from the emergency medical response system, the medical examiner network and 16 local hospitals. All available medical records were obtained for each subject following the SCD event, providing a detailed lifetime clinical history for the purpose of phenotyping. SCD was defined as an unexpected pulseless condition of likely cardiac origin. If unwitnessed, SCD was defined as unexpected death within 24 h of having last been seen alive and in a normal state of health [3]. CAD was defined as 50% stenosis of a major coronary artery, physician report of past myocardial infarction (MI), history of percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG); autopsy-identified CAD or MI by clinical data with any two of the following three: ischemic symptoms, positive troponins or CKMB; or pathologic Q waves on ECG. SCD cases with chronic terminal illnesses, known non-cardiac causes of SCD, traumatic deaths and drug overdose were excluded from the analysis.

2.2.2. WTCCC subjects

A total of 1926 coronary artery disease (CAD) subjects were included as the comparison (control) group. We used CAD subjects rather than population controls due to the significant overlap between SCD and CAD (CAD is diagnosed in at least 80% of overall SCD cases) [29]. This would maximize the chances of developing a GRS that would be specific for SCD. CAD status and diagnosis was validated by direct review of clinical notes. All subjects were of White European origin and further details of the WTCCC subjects were previously reported [30].

2.2.3. Genotyping and SNP selection

The Oregon-SUDs cases and the WTCCC controls were genotyped and imputed separately using Affymetrix 6.0 and 500 K arrays respectively. SNPs were selected based on information obtained from the NHGRI GWAS Catalogue [31] and literature review [14–25]. We selected 55 independent SNPs previously identified in GWAS studies for SCD-related phenotypes with $P = 5 \times 10^{-8}$, these include: QRS duration, QT interval, and heart rate. (Table 1 and Supplementary Table 1). We also included 14 SNPs associated with SCD in previous GWAS and candidate gene studies [14–22]. Deviation from Hardy Weinberg equilibrium was not observed for any SNP in either cases or controls. Three SNPs with minor allele frequency less than 5% were excluded from further analyses. After quality control, 66 SNPs for the 4 traits were included. For imputed SNPs, we used the posterior continuous values between 0 and 2. A complete list of the investigated variants is provided in Supplementary Table 1.

2.2.4. Statistical analysis

Separate GRSs were developed for SNPs previously associated with increased risk of SCD (14 SNPs), prolonged QRS duration (26 SNPs), QT interval (9 SNPs) and increased heart rate (18 SNPs). For each of these GRSs, a weighted GRS was calculated for each individual based on the number of risk alleles weighted by the standardized beta coefficients derived from the original GWAS studies. Genetic risk scores were tested for association by using logistic regression models implement in Golden Helix SNP and Variation Suite (Golden Helix, Bonzeman, MT, USA). The status (case–control) was used as the outcome variable and the GRS as the predictor variable. In addition, we analyzed associations between individuals in the lowest and highest quintiles for the GRS composed of SCD SNPs using logistic regression. Individuals in the highest quintile were classified as high risk. Analyses were adjusted for age and gender.

3. Results

The mean ages of the subjects in the Oregon-SUDS and the WTCCC studies were 60.3 ± 9.4 and 49.7 ± 12.8 years respectively. Seventy two percent of SCD cases and 79.2% of CAD controls were male. SCD cases were relatively older than CAD controls and therefore we performed analyses using the complete sample size in the Oregon-SUDs (n = 966) as well as a sample size restricted to the same age range observed in WTCCC (n = 668); similar results were observed (Table 2).

3.1. Genetic risk score associations

We investigated association of each of the weighted GRS in the merged Oregon-SCD cases and WTCCC CAD control dataset including a total of 2892 unrelated individuals.

The cumulative effects of 14 common variants previously involved in SCD was modestly associated with an increased risk (OR = 1.17, P = 0.002). The results remained significant after adjusting for multiple testing comparisons. The risk for SCD was 1.5 fold greater in the highest risk quintile when compared to the lowest risk quintile (OR = 1.46 [1.11–1.92]). Two DNA variants within Calsequestrin 2 (*CASQ2*),

Table 1
Traits included for the genetic risk scores estimations.

Traits	No. of SNPs	Reference
SCD[a]	14	14–22
QRS duration	24	23
Heart rate	19	24
QTc interval	9	25

[a] Sudden cardiac death.

Table 2
Association of GRS with SCD associated phenotypes.

		966 cases/1926 controls[a]		668 cases/1926 controls[b]	
Phenotype	N SNPs	OR (95% CI)[c]	P-value	OR (95% CI)[c]	P-value
Heart rate	18	0.59 [0.24–1.41]	0.23	0.56 [0.22–1.41]	0.22
QTc interval	9	1.01 [0.49–2.09]	0.69	0.86 [0.36–2.01]	0.73
QRS duration	26	1.04 [0.87–1.24]	0.65	1.06 [0.88–1.27]	0.51
SCD[d]	14	1.17 [1.05–1.29]	0.002	1.16 [1.05–1.29]	0.003

[a] Results including 966 SCD cases and 1926 controls from the WTCCC.
[b] Results including a subset of 668 SCD cases (age range 26–67 years) and 1926 controls from the WTCCC.
[c] OR indicates Odds ratio (95% confidence interval) per weighted allele increase in the GRS for each investigated trait. Data was adjusted by age and gender.
[d] Sudden cardiac death.