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

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Background—Recent evidence suggests a genetic component for sudden cardiac death (SCD) in subjects with coronary artery disease (CAD). We conducted a systematic candidate-gene approach using haplotype-tagging single nucleotide polymorphisms (htSNPs) to identify genes associated with SCD risk in the context of CAD.

Methods and Results—We investigated 1424 htSNPs representing 18 genes with mutations described in patients with ventricular arrhythmias in 291 subjects from the Oregon Sudden Unexpected Death Study (Ore-SUDS). The Ore-SUDS is an ongoing prospective investigation of SCD in the Portland, OR, metropolitan area (population, 1 000 000). SCD cases were ascertained from multiple sources and medical records were reviewed to determine the presence of CAD. A total of 36 SNPs were associated with risk of SCD (uncorrected probability values <0.01) in the initial study sample. These SNPs were subsequently tested for replication in an independent case-control study sample from the Ore-SUDS (n=688). The association analysis in the replication stage revealed 6 SNPs associated with SCD: CASQ2 region (rs17500488, \(P=0.04\); rs3010396, \(P=0.007\); rs7366407; \(P=0.04\)), NOS1AP (rs12084280, \(P=0.04\); rs1091859, \(P=0.02\)), and 1 SNP located 26 kb upstream of GPD1L (rs9862154, \(P=0.04\)).

Conclusions—Common variations in or near CASQ2, GPD1L, and NOS1AP are associated with increased risk of SCD in patients with CAD. These findings provide further evidence for overlap between the genetic architecture of rare and common forms of SCD, and replication in additional populations is warranted. (Circ Cardiovasc Genet. 2011;4:397-402.)

Key Words: death, sudden ■ risk prediction ■ genomics ■ variants

Sudden cardiac arrest leading to sudden cardiac death (SCD) is a major cause of mortality in the United States, accounting for 250 000 to 300 000 deaths on an annual basis.\(^1\) Prediction and prevention have been recognized as pivotal steps toward improved outcomes,\(^1\) particularly because national rates of survival from sudden cardiac arrest are below 5%. Because the vast majority of SCD cases (at least 80%) have evidence of associated severe coronary artery disease (CAD),\(^2\) the latter phenotype has become the focus of intensive investigation.

Clinical Perspective on p 402

Several studies have highlighted the evidence for a clear genetic contribution in the more common SCD phenotype among patients with coronary artery disease.\(^3\) We have recently published results from ongoing genome-wide association studies of SCD, identifying novel loci associated with either protection from,\(^7\) or susceptibility to SCD.\(^8\) However, much must be learned regarding the genetic architecture of common, complex forms of SCD.\(^9\) On the other hand, due to multiple kindred-based investigations performed in the last 2 decades, significant knowledge has accumulated regarding gene defects that cause rare primary arrhythmia syndromes. Several mutations in multiple genes have been identified in inherited forms of the long- and short-QT syndromes.\(^10\) Brugada syndrome is characterized by idiopathic ventricular fibrillation and characteristic ECG changes,\(^15\) and catecholaminergic polymorphic ventricular tachycardia is a familial arrhythmogenic disorder manifesting with ventricular tachyarrhythmias.\(^18\) Primary arrhythmia syndromes account for only a small proportion of SCD cases in the general population. We hypothesized that variations in genes that cause primary arrhythmia syndromes could be associated with the more common, complex SCD phenotype observed in patients with CAD. Given that candidate gene-based evaluations can be complementary to genome-wide association efforts, we conducted a systematic candidate-gene SNP genotyping, case-control study of SCD in CAD subjects.
based on common variations among genes known to cause primary arrhythmia disorders.

Methods

Clinical Diagnosis

The Oregon Sudden Unexpected Death Study (Ore-SUDS) is an ongoing population-based study of SCD residents of Portland, OR, and the surrounding metropolitan area.7,20–23 SCD was defined as a sudden unexpected pulseless condition of likely cardiac etiology; survivors of sudden cardiac arrest were included. If the event was witnessed, subjects were required to have been seen living and symptom free within 24 hours of sudden death. A diagnosis of SCD was assigned after in-house adjudication by 3 physicians who evaluated arrest circumstances and all available clinical data. Exclusion criteria for Ore-SUDS SCD cases were chronic terminal illness and noncardiac causes of sudden death such as pulmonary embolism, cerebrovascular event, traumatic death, or drug overdose.

Subject Selection

Case subjects in this analysis were individuals with SCD who were white, non-Hispanic adults (age ≥18 years) with DNA for analysis. Control subjects were white, non-Hispanic individuals with medically documented CAD, and without prior history of sudden cardiac arrest or ventricular arrhythmias. They were recruited from individuals transported by the Emergency Medical Response system for complaints suggestive of ongoing coronary ischemia, from clinics of participating physicians, or from patients who had received a coronary angiogram or coronary artery bypass grafting; physician report of myocardial infarction; pathological Q waves on ECG; or myocardial infarction history determined by any 2 of the following 3: ischemic symptoms, ECG changes, or positive troponins/creatine kinase MB. All control subjects had documented CAD. A total of 346 SCD cases (52%) had documented CAD by autopsy or medical records. Ninety-four percent of cases had either medically documented CAD, as defined below, or had presumed CAD, based on previous studies that have reported that the vast majority (85% to 95%) of subjects with SCD at age ≥50 years have significant coronary disease at autopsy.2–26

Blood samples were obtained for SCD cases from the first responders during attempted resuscitation or from the medical examiner, when autopsies were performed. Samples were obtained from control subjects at the time of their visit to the study site for a blood draw and ECG. All aspects of this study were approved by the appropriate institutional review boards.

Candidate-Based Genotyping and SNP Selection

We performed a custom SNP genotyping assay on the initial study population using a candidate-gene–based approach with the GoldEnGate assay (Illumina, Inc, San Diego, CA). SNPs representing 18 high-priority genes were selected for analysis. Genes were considered to have a high priority if mutations had been described in patients with primary ventricular arrhythmia syndromes or if these genes were encoding crucial subunits of such candidate genes. A list of the selected genes is shown in Table 1.

The common genetic variation of each gene was covered by systematic selection of haplotype tagging single nucleotide polymorphisms (htSNPs), considering both intronic and exonic variants. SNP selection was performed in mid 2007 and was performed using the software tagger,27 based on the HapMap data release No. 20/phase II from January 2006, using the NCBI B35 genome assembly and dbSNP b125 data and applying the following criteria: HapMap CEU population, pairwise tagging only with a cutoff of r²≥0.8, and a minor allele frequency of at least 10%. To account for genetic variation in genome regions surrounding each gene, upstream and downstream genetic information was included in the tagging procedure. The respective regions were defined using linkage-disequilibrium (LD) blocks as described elsewhere.28 If the detected LD blocks were smaller than 50 kb, then at least 50 kb of both upstream and downstream information was tagged. Due to the potentially higher a priori probability of being a pathophysiological-

### Table 1. Candidate Genes Under Investigation and Number of Tag SNPs Genotyped

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chr</th>
<th>Position</th>
<th>Region Included</th>
<th>No. of SNPs</th>
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<td>7</td>
<td>91570192-91739989</td>
<td>9330760-9396760</td>
<td>20</td>
</tr>
<tr>
<td>ANK 2 Isoform 1</td>
<td>4</td>
<td>113970870-114304885</td>
<td>113802396-11432396</td>
<td>118</td>
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<td>CADNA1C</td>
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<td>2162464-2802107</td>
<td>2029739-2949739</td>
<td>176</td>
</tr>
<tr>
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<td>162039581-162339813</td>
<td>161948342-162438342</td>
<td>130</td>
</tr>
<tr>
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<td>116097958-116417958</td>
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<tr>
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<td>FKB1P2</td>
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<td>24272628-24286548</td>
<td>24222627-24363547</td>
<td>13</td>
</tr>
<tr>
<td>GPD1L</td>
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<td>32004996-32254996</td>
<td>50</td>
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<td>35718130-36008130</td>
<td>71</td>
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<td>35718130-36008130</td>
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<td>KCNH2/HERG</td>
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<td>50152739-150762352</td>
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<tr>
<td>KCNJ2</td>
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<td>68098405-68253405</td>
<td>42</td>
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<tr>
<td>KCNO1</td>
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<td>2466221-2870339</td>
<td>2303424-2943424</td>
<td>151</td>
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<td>237205702-237997288</td>
<td>237101177-238101176</td>
<td>240</td>
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<tr>
<td>SCN1B</td>
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<td>35521534-35531352</td>
<td>3548160-3578160</td>
<td>24</td>
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<tr>
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<td>35895553-38691163</td>
<td>33867996-38814996</td>
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</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>1424</td>
</tr>
</tbody>
</table>

SNP indicates single nucleotide polymorphism; Chr, chromosome.

All data are based on hg 19, NCBI build 37.
ly causal variant, all known nonsynonymous coding variants in the selected genes were added to the assay design.

**SNP Genotyping**

A SNP genotyping assay containing all mentioned variants was purchased from Illumina, based on the Illumina GoldenGate technology. Genotyping was performed according to the manufacturer’s recommendations using the Illumina Beadstation 500G. Illumina BeadStudio 3.1.14 genotyping module was used to automatically cluster, call genotypes, and assign confidence scores. All markers with call frequency lower than 95% were manually edited.

**SNP Validation**

SNPs significantly associated with risk of SCD were subsequently validated on a different set of cases and control subjects. These samples from the Ore-SUDS study were also white, Non-Hispanic from the same geographic area as the original sample and ascertained according to the subject selection characteristics listed above. Genotyping for the replication stage was performed using polymerase chain reaction, iPLEX single base primer extension, and subsequent matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) mass spectrometry on a Sequenom platform (Sequenom, San Diego, CA), according to the manufacturer’s standard recommendations. A total of 40 SNPs were processed in 2 experiments of 30 and 10 variants, respectively. Replicated associations with a Pearson χ² probability value <0.05 were considered truly associated with the phenotype. SNP rs9862154 did not meet the call rate cutoff in the iPLEX and was genotyped in a single ABI TaqMan Genotyping assay according to the manufacturer’s directions (Applied Biosystems, Inc.).

**Statistical Analysis**

Association analyses were performed on the original study population and the validation dataset, using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/). SNPs were tested for genotype-phenotype association using the genotypic C/C association test in PLINK, which calculates the association of genotype to SCD using a full model of inheritance. Asymptotic probability values are provided for each of four association tests: additive, genotype, dominant, and recessive.

**Results**

**Genotyping in the Discovery Population**

A panel of 1424 SNPs was genotyped in 291 individuals. The average call rate was 96%.

Our initial study population consisted of 141 cases (43 female and 98 male) and 150 control subjects (48 female and 102 male). Ten individuals missing >10% genotype data were removed from analysis. The sample remaining for analysis consisted of 134 cases (mean age, 66±14 years; 71% male) and 147 control subjects (mean age, 66±13 years; 67% male) (Table 2).

Of 1424 SNPs, 50 SNPs were missing >10% of genotypes and 67 SNPs had an minor allele frequency of <0.01, which resulted in exclusion of a total of 76 SNPs from the dataset. SNPs not in Hardy-Weinberg equilibrium (P<0.001) in control subjects (n=14) were removed from the study because their inclusion could lead to false signals of association. Thus, 281 subjects with genotypes in 1334 SNPs (overall call rate of 99.64%) were included in the final dataset. Thirty-eight SNPs were associated with SCD (uncorrected probability value <0.01) in at least 1 of the association models (Table 3), whereas 53 significant associations would have been expected by chance alone.

**Genotyping in the Validation Population**

In the second stage, we performed validation genotyping for these 38 SNPs in an independent Ore-SUDS sample (n=688). Replicated associations with a Pearson χ² probability value statistic of <0.05 were considered truly associated with the phenotype. Several SNPs showed association with SCD on chromosome 1, near the CASQ2 gene (just upstream of NOS1AP) and in the NOS1AP gene (nitric oxide synthase 1 adaptor protein) under different genetic models. In addition, the SNP rs9862154 ≈26 kb upstream of GPD1L was associated with SCD under the recessive genetic model (Table 3).

**Discussion**

In the present study, we observed and validated significant associations between DNA variants located in noncoding regions of CASQ2, GPD1L, and NOS1AP genes and risk of SCD in subjects with CAD. CASQ2 and GPD1L are of special interest because of their known involvement in the primary arrhythmia syndromes, and NOS1AP has been previously associated with prolongation of the QT interval and risk of SCD in the community. These findings indicate the interesting possibility of overlap between the genetic architecture of rare and common forms of SCD. The potential for false-positive results should be evaluated in future, larger replication efforts.

CASQ2 encodes the intrasarcoplasmic reticulum Ca²⁺-binding protein cardiac calsequestrin. Mutations in CASQ2 have been associated with catecholaminergic polymorphic ventricular tachycardia, a rare familial arrhythmogenic disorder characterized by malignant ventricular arrhythmias. GPD1L can harbor coding mutations among kindreds with the Brugada syndrome. An interesting relationship has also been described between GPD1L and the SCN5A gene, implicated in the majority of causative mutations discovered for Brugada syndrome. London et al. have shown that missense mutations in GPD1L cause reduced trafficking of the cardiac Na⁺ channel to the cell surface, reducing inward Na⁺ current, and causing Brugada syndrome. Furthermore, the downregulation of Na current by GPD1L mutations probably is due to alteration of the oxidized to reduced nicotinamide adenine dinucleotide hydrogenase [NAD(H)] balance. In recent work, Valdivia et al. lends support for linking mutations in GPD1L to SCD, using an in vitro cell culture system expressing GPD1L and SCN5A mutant and wild-type constructs. They demonstrated association of GPD1L with SCN5A; mutants of GPD1L increased protein kinase C–mediated phosphorylation of SCN5A, which in turn causes a dysfunction in sodium current, a mechanism for ventricular arrhythmias.

NOS1AP encodes a nitric oxide synthase 1 adaptor protein. Common variants in NOS1AP have been associated with...
prolongation of the QT interval as well as increased risk of SCD. Kao et al reported that 2 noncorrelated NOS1AP SNPs (rs16847548 and rs12567209) were associated with SCD in a large US community. The SNP rs12567209 is in high LD with rs12084280 reported in the present study (D\(^2\) = 1.0; r\(^2\) = 0.92). Of note, a NOS1AP variant was also identified as a risk modifier among patients with familial long-QT syndrome. Although it is well documented that NOS1AP common variants are associated with increased risk of SCD, the specific functional role of NOS1AP variants merits further evaluation.

Whereas mutations have been described and characterized in CASQ2 earlier, in the present study we report a role for common variants for which functional evaluation has yet not been performed. One hypothesis might be that common SNPs are markers of functional, rare variants that are not covered by current genotyping strategies, similar to what has been shown for hypertriglyceridemia.

Table 3. SNPs Significantly Associated (P<0.01) With SCD

<table>
<thead>
<tr>
<th>Chr</th>
<th>SNP</th>
<th>MAF</th>
<th>Gene</th>
<th>Model</th>
<th>P Value*</th>
<th>Validation P Value</th>
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<td>1</td>
<td>rs17500488</td>
<td>0.095</td>
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<td>1</td>
<td>rs7536370</td>
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<td>CASQ2</td>
<td>DOM</td>
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<td>0.800</td>
</tr>
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<td>GENO</td>
<td>0.0208</td>
<td>0.007†</td>
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<tr>
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<td>rs11586273</td>
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<td>0.0053</td>
<td>0.120</td>
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<tr>
<td>1</td>
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<td>0.0023</td>
<td>0.040†</td>
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<td>GENO</td>
<td>0.0028</td>
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</tr>
</tbody>
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SNP indicates single nucleotide polymorphism; Chr, chromosome; ADDITIVE, additive genetic model; DOM, dominant genetic model; GENO, genotypic model; REC, recessive genetic model; and MAF, minor allele frequency in all subjects. *P value from additive, dominant, recessive, or genotypic model. †Validation P<0.05.
relationship between common and rare variants at a single locus and to identify pathophysiologically causal variants, the current advent of high-throughput sequencing efforts is a promising strategy for the near future.

Limitations
Our sample size is relatively small, and the possibility exists that we have missed additional association signals. Future investigation in larger cohorts will be needed to detect such associations with sufficient statistical power. However, this is a challenging phenotype to study in the community and subjects were matched for presence of CAD. Furthermore, our cases and control subjects were all derived from the same underlying population and adjudicated by following a common, standardized protocol. All tested genes bear a high a priori probability for a true association, based on previous reports on their pathophysiological involvement in our phenotype. A Bonferroni correction for multiple testing is often applied in genome-wide association studies but might be considered too conservative for highly selective candidate gene-based approaches. Independent replication of significant findings can be regarded as the most reliable form of validation. We therefore did not perform correction for multiple testing in association results in either the discovery or the replication population but rather attempted independent replication.

Conclusions
These findings suggest that common variants in genes previously implicated in relatively rare inherited forms of arrhythmias may contribute to the pathogenesis of more common, complex forms of SCD. Further studies in larger samples are warranted to validate the contribution of these genes in SCD.

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Disclosures
None.

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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.
Common Variants in CASQ2, GPD1L, and NOS1AP Are Significantly Associated With Risk of Sudden Death in Patients With Coronary Artery Disease
Shawn K. Westaway, Kyndaron Reinier, Adriana Huertas-Vazquez, Audrey Evanado, Carmen Teodorescu, Jo Navarro, Moritz F. Sinner, Karen Gunson, Jonathan Jui, Peter Spooner, Stefan Kaab and Sumeet S. Chugh

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