The Contribution of Known Familial Cardiovascular Disease Genes to Sudden Cardiac Death in Patients Undergoing Hemodialysis

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Cardiovascular disease · Sudden cardiac death · Arrhythmia · Dialysis · Kidney

Abstract
Introduction: Patients with chronic kidney disease experience high rates of cardiovascular mortality and morbidity. When kidney disease progresses to the need for dialysis, sudden cardiac death (SCD) accounts for 25–35% of all cardiovascular deaths. The objective was to determine if rare genetic variants known to be associated with cardiovascular death in the general population are associated with SCD in patients undergoing hemodialysis. Methods: We performed a case-control study comparing 126 (37 African American [AfAn] and 89 European ancestry [EA]) SCD subjects and 107 controls (34 AfAn and 73 EA), matched for age, sex, self-reported race, dialysis duration (<2, 2–5, and >5 years), and the presence or absence of diabetes mellitus. To target the coding regions of genes previously reported to be associated with 15 inherited cardiac conditions (ICCs), we used the TruSight Cardio Kit (Illumina, San Diego, CA, USA) to capture the genetic regions of interest. In all, the kit targets 572-kb regions that include the protein-coding regions and 40-bp 5’ and 3’ end-flanking regions of 174 genes associated with the 15 ICCs. Using the sequence data, burden tests were conducted to identify genes with an increased number of variants among SCD cases compared to matched controls. Results: Eleven genes were associated with SCD, but after correction for multiple testing, none of the 174 genes were identified as having more variants in the SCD cases than the matched controls, including previously identified genes. Secondary burden tests grouping variants based on diseases and gene function did not produce statistically significant associations. Discussion/Conclusions: We found no associations between genes known to be associated with ICCs and SCD in our sample of patients undergoing hemodialysis. This suggests that genetic causes are unlikely to be a major pathogenic factor in SCD in hemodialysis patients, although our sample size limits definitive conclusions.

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Introduction
Cardiovascular disease is the major cause of death in patients with chronic kidney disease (CKD). When patients have progressive CKD and require dialysis (end-
stage kidney disease [ESKD]), cardiovascular manifestations change from a predominant atherosclerotic disease to a left ventricular hypertrophy and heart failure with preserved ejection fraction. In patients with ESKD, diastolic dysfunction, non-ST elevation myocardial infarction, and bradyarrhythmias are more common than systolic dysfunction, ST elevation infarction, and ventricular arrhythmias [1–3]. Sudden cardiac death (SCD) accounts for 25–35% of all cardiovascular deaths in patients with ESKD in both adjudicated clinical trials [4] and in association studies of large databases [5, 6]. The dialysis procedure had been assumed to cause SCD due to rapid volume and electrolyte shifts. However, studies also show an increased risk of SCD with progressive CKD, often long before dialysis is required (reviewed in [7]), suggesting increased risk of SCD with progressive CKD, often long before dialysis is required (reviewed in [7]), suggesting increased risk of SCD with progressive CKD, often long before dialysis is required (reviewed in [7]), suggesting increased risk of SCD with progressive CKD,

A study of 47 patients with ESKD and a prolonged QT interval found 5 genes associated with arrhythmias; in the follow-up, 2 patients died of SCD [9]. We previously reported that 3 correlated single-nucleotide polymorphisms (SNPs) in the angiotensin-converting enzyme (ACE) gene were associated with lower rates of SCD in European Americans enrolled in the Effect of Cinacalcet on Cardiovascular Disease in Patients Undergoing Dialysis (EVOLVE) trial. One ACE SNP, rs4318, only found in the African American sample, was associated with a higher rate of SCD [10]. Thus, there is some evidence that genetics may be important in SCD in patients with ESKD. However, the association of rare genetic variants, especially those associated with SCD in the general population [11], has not been examined in patients on dialysis. Given the disproportionate frequency of SCD in ESKD, we examined these variants in a case-control study using DNA and adjudicated endpoints from the EVOLVE trial [12, 13].

**Materials and Methods**

**Participant Selection**

The EVOLVE trial randomized 3,883 patients with ESKD receiving hemodialysis thrice weekly with moderate to severe secondary hyperparathyroidism to either cinacalcet or placebo in addition to standard-of-care treatments which typically included phosphate binders and calcitriol (1,25-dihydroxy vitamin D) or other active vitamin D analogs. An independent clinical events committee adjudicated all primary and secondary endpoints, including SCD [13]. The primary endpoint of the EVOLVE trial was a composite of all-cause mortality, nonfatal myocardial infarction, hospitalization for unstable angina, heart failure, and peripheral vascular event. A post hoc analysis [4] found that 54% of all deaths in the EVOLVE trial were due to cardiovascular disease, and of those, nearly 1 in 4 (352 patients) was due to SCD, whereas only 4% of deaths were due to acute myocardial infarction. Of the 3,883 EVOLVE participants, 1,919 (49%) consented to have DNA collected (confirmed internally at Amgen), and 1,852 samples were of adequate quality to be genotyped, and the project was approved by the Indiana University Institutional Review Board [14]. Of those samples with DNA, we identified 126 patients with SCD (37 African American [AfAn] and 89 European ancestry [EA] samples) and then selected 107 controls (34 AfAn and 73 EA) who were matched for age, sex, self-reported race, dialysis duration (<2, 2–5 and >5 years), and the presence or absence of diabetes for sequencing.

**DNA Sequencing**

DNA was extracted from white cell pellets or peripheral blood obtained from the 233 participants (126 cases and 107 controls) who were chosen for sequencing. To target the coding regions of genes previously reported to be associated with 15 inherited cardiac conditions (ICCs: long QT syndrome [LQTS], short QT syndrome [SQTS], Brugada syndrome [BrS], catecholaminergic polymorphic ventricular tachycardia [CPVT], hypertrophic cardiomyopathy [HCM], dilated cardiomyopathy [DCM], arrhythmogenic right ventricular cardiomyopathy [ARVC], restrictive cardiomyopathy [RCM], left ventricular noncompaction [LVNC], Noonan syndrome, Marfan syndrome [MFS], Loep–Dietz syndrome [LDS], familial aortic aneurysm, aortic valve disease, and familial hypercholesterolemia), we used the TruSight Cardio Kit (Illumina, San Diego, CA, USA) to capture the coding regions of the reported genes. In all, the kit targets 372-kb regions that include the protein coding regions and 40–50’ 5’ and 3’ end-flanking regions of 174 genes associated with the 15 ICCs (genetic details of the genes in the supplement of [15]). Sequencing was conducted at the IU Molecular Genetics Diagnostic Laboratory (IUMGL) at the Indiana University School of Medicine (IUSM) using MiSeq (Illumina, San Diego, CA, USA) following the manufacturer’s procedures.

**Sequence Data Processing**

We processed sequence data using the in-house NextGen sequence data pipeline that uses Genome Analysis Tool Kit version 4 [16] following the best practices [17] guideline. In brief, reads were first mapped to human reference genome build 38 using BWA [18]. We then sorted, de-duplicated, and recalibrated base quality scores from mapped reads using Genome Analysis Tool Kit version 4. Using HaplotypeCaller, we identified variants in each individual separately, resulting in a gvcf file for each EVOLVE participant. Those gvcf files were then combined for group-level variant calling. The resulting file was a set of SNPs and insertion/deletions. Due to the limited number of samples and limited size of sequenced regions, we filtered variants based on the parameters (below), instead of the more typical variant quality score recalibration procedure in GATK. We required the following: (a) quality by depth ≥2.0, (b) Fisher strand ≥60.0, (c) root-mean-square of the
mapping quality of the reads across all samples ≥40.0, (d) mapping quality rank sum test statistic ≥−12.5, (e) read position rank sum test statistic ≥8.0, and (f) strand odds ratio ≤3.0 for SNPs in the vcf file. For the insertion/deletions, we required the following: (a) quality by depth ≥2.0, (b) Fisher strand ≤200.0, (c) read position rank sum test statistic ≥20.0, and (d) strand odds ratio ≤3.0. After filtering, we annotated all variants that passed QC using ANNOVAR [19].

### Variant Selection for Analysis

Variants passing QC, as described earlier, were filtered based on their annotated functions and minor allele frequencies (MAFs) from gnomAD [20] prior to burden testing. First, we kept those SNPs that were predicted to change the amino acid sequence (coding change) based on the annotated functions, including frameshift insertion, frameshift deletion, nonsynonymous SNV, stopgain, and stoploss. For those variants predicted to be functional, we removed those with MAFs ≥5% based on reported population-level frequencies from gnomAD non-Finnish European and African samples. After filtering for predicted function (amino acid coding changing) and population-based MAFs (MAF <5%), AfAn samples had 1,514 variants and EA samples had 1,610 variants to be used in the analyses in 153 genes.

### Statistical Analysis

Clinical and laboratory characteristics of patients with SCD were compared (to the entire EVOLVE cohort after removing those SCD cases) by using the χ² test for categorical items and the 2 sample t test for continuous items. After post hoc adjustment based on the 28 comparisons, a p value of 0.0018 was re-

### Table 1. Baseline demographics and laboratory values

|                          | SCD cases, n = 126 | SCD controls, n = 107 | EVOLVE cohort, n = 3,883 |
|--------------------------|-------------------|-----------------------|--------------------------|
| Cinacalcet use, n (%)    | 60 (48)           | 52 (49)               | 1,948 (50)               |
| Age, years, mean (SD)    | 59.7 (11.5)       | 59.6 (11.8)           | 54.4 (14.4)*             |
| Sex (female), n (%)      | 51 (40)           | 44 (41)               | 1,578 (41)               |
| **Ethnicity (self-reported), n (%)** |                     |                       |                          |
| European ancestry       | 89 (71)           | 73 (68)               | 2,240 (58)               |
| African ancestry        | 37 (29)           | 34 (32)               | 837 (22)                 |
| Other                   | 0 (0)             | 0 (0)                 | 806 (21)                 |
| **Dialysis vintage, n (%)** |                     |                       |                          |
| <2 years                | 36 (29)           | 31 (29)               | 1,098 (28)               |
| 2 to <5 years           | 45 (36)           | 37 (35)               | 1,285 (33)               |
| ≥5 years                | 45 (36)           | 39 (36)               | 1,499 (39)               |
| Current or previous smoker, n (%) | 58 (46)       | 53 (50)               | 1,696 (44)               |
| Abnormal baseline ECG, n (%) | 92 (73)         | 70 (65)               | 2,263 (58)               |
| Diabetes (type 1 or type 2), n (%) | 64 (51)       | 52 (49)               | 1,302 (34)*              |
| Chronic atrial fibrillation, n (%) | 13 (10)       | 13 (12)               | 227 (6)                  |
| Arrhythmia of any type, n (%) | 33 (26)       | 23 (21)               | 554 (14)+                |
| Hypertension, n (%)      | 118 (94)          | 101 (94)              | 3,577 (92)               |
| Coronary artery bypass graft, n (%) | 15 (12)     | 11 (10)               | 289 (7)                  |
| Myocardial infarction, n (%) | 19 (15)        | 17 (16)               | 483 (12)                 |
| Heart failure, n (%)     | 44 (35)           | 36 (34)               | 906 (23)                 |
| Stroke, n (%)            | 14 (11)           | 13 (12)               | 355 (9)                  |
| Peripheral arterial disease, n (%) | 28 (22)     | 27 (25)               | 635 (16)                 |
| Amputation, n (%)        | 8 (6)             | 13 (12)               | 250 (6)                  |
| **Baseline drug use, n (%)** |                     |                       |                          |
| Calcium-based phosphate binders | 70 (56)   | 49 (46)               | 2,062 (53)               |
| Baseline vitamin D       | 75 (60)           | 78 (73)               | 2,310 (59)               |
| **Baseline laboratory values** |                     |                       |                          |
| Corrected Ca, mean (SD), mg/dL | 9.8 (0.7)   | 9.9 (0.7)             | 9.8 (0.7)                |
| Phosphorus, mean (SD), mg/dL | 6.6 (1.7)   | 6.3 (1.2)             | 6.5 (1.4)                |
| Intact PTH, median (p10, p90), pg/mL | 610 (356, 1,498)| 597 (368, 1,281) | 693 (363, 1,694)         |
| FGF23, median (p10, p90), pg/mL | 4,380 (412, 23,914)| 4,465 (600, 15,095) | 5,590 (580, 19,528)      |
| Potassium, mean (SD), mg/dL | 5.1 (0.8)   | 4.9 (0.7)             | 5.1 (0.8)                |
| Hemoglobin, mean (SD), mg/dL | 11.8 (1.2)  | 12 (1.4)              | 11.8 (1.5)               |
| Total cholesterol, mean (SD), mg/dL | 159.8 (41.1) | 160.8 (40.8)         | 166.8 (43.4)             |
| HDL cholesterol, mean (SD), mg/dL | 43.8 (15.7) | 44.1 (14.3)          | 43.4 (15)                |
| Triglycerides, mean (SD), mg/dL | 155.6 (116.6) | 162.6 (114)          | 169.3 (120.3)            |

+ p < 0.0018 SCD versus entire EVOLVE cohort.
### Table 2. Gene level burden tests

| Gene symbol | Gene name/associated cardiac syndrome* | Combined variants, n | Odds ratios† | p value |
|-------------|----------------------------------------|-----------------------|--------------|---------|
| APOA4       | Apolipoprotein A4/FH                    | 8                     | 0.40         | 0.006   |
| CASQ2       | Caldesmon 2 (cardiac muscle)/CPVT       | 2                     | 0.10         | 0.015   |
| APOE        | Apolipoprotein E/FH                     | 2                     | 0.12         | 0.017   |
| ABCG9       | ATP-binding cassette, subfamily C (CFTR/MPR), member 9/BrS | 3                     | 4.51         | 0.020   |
| LDLR        | Low-density lipoprotein receptor/FH     | 12                    | 0.59         | 0.024   |
| SNTA1       | Synaptophysin, alpha 1 (dystrophin-associated protein A1, 59 kDa, acidic component)/LQTS | 7                     | 0.32         | 0.031   |
| DES         | Desmin/HCM                              | 8                     | 0.35         | 0.039   |
| TBX3        | T-box transcription factor TBX3/LQTS    | 4                     | 2.07         | 0.040   |
| ANKRD1      | Ankyrin repeat domain 1 (cardiac muscle)/HCM | 5                     | 0.23         | 0.043   |
| HFE         | Hemochromatosis/DCM                     | 5                     | 1.71         | 0.045   |
| ABCG5       | ATP-binding cassette (ABC) transporters G5/FH | 10                    | 0.50         | 0.047   |
| ZHX3        | Zinc fingers and homeoboxes protein 3/DCM | 10                   | 0.60         | 0.054   |
| GATAD1      | GATA zinc finger domain containing 1/DCM | 2                     | 3.43         | 0.054   |
| JAG1        | Jagged 1 (ligand in notch pathway)/FAA  | 7                     | 1.91         | 0.057   |
| CAV3        | Caveolin 3/LQTS                         | 3                     | 3.12         | 0.060   |
| MYLK        | Myosin light chain kinase 2/DCM        | 28                    | 1.18         | 0.075   |
| LMNA        | Lamin A/C/DCM                           | 2                     | 0.14         | 0.077   |
| TAZ         | Tafazzin/DCM                            | 2                     | 0.25         | 0.080   |
| TXNRD2      | Thioredoxin reductase 2/DCM             | 6                     | 2.29         | 0.091   |
| ZBTB17      | Zinc finger and BTB domain-containing protein 17/DCM | 2                     | 0.31         | 0.101   |
| HSPB8       | Heat shock protein B8 (aB-crystallin)/DCM | 2                     | 6.08         | 0.111   |
| LPL         | Lipoprotein lipase/FH                   | 7                     | 0.46         | 0.114   |
| SGCB        | Sarcoglycan, beta (43 kDa dystrophin-associated glycoprotein)/DCM | 2                     | 0.10         | 0.120   |
| KCND3       | Potassium voltage-gated channel, Shal-related subfamily, member 3/BrS | 4                     | 0.24         | 0.125   |
| LTP2        | Latent transforming growth factor beta binding protein 2/DCM | 21                    | 0.59         | 0.133   |
| KCNJ5       | Potassium inwardly rectifying channel, subfamily J, member 5/LQTS | 2                     | 0.27         | 0.137   |
| ELN         | Elastin/AVD                             | 12                    | 1.54         | 0.138   |
| AP0C2       | Apolipoprotein C2/FH                    | 6                     | 3.10         | 0.144   |
| NKX2-5      | NKX2 homeobox 5/DCM                     | 2                     | 0.43         | 0.154   |
| CACNA2D1    | Calcium channel, voltage-dependent, alpha 2/delta subunit 1/SQTS | 4                     | 1.61         | 0.158   |
| PCSK9       | Proprotein convertase subtilisin/kexin type 9/FH | 15                    | 1.32         | 0.186   |
| ANK2        | Ankyrin 2, LQT                          | 31                    | 0.82         | 0.188   |
| TPM1        | Tropomyosin 1 (alpha)/HCM              | 3                     | 5.91         | 0.203   |
| TNNT2       | Troponin T type 2 (cardiac)/DCM        | 3                     | 1.46         | 0.208   |
| FXN         | Frataxin/DCM                            | 3                     | 0.54         | 0.228   |
| VCL         | Vinculin/DCM                            | 4                     | 0.29         | 0.235   |
| COL5A2      | Collagen type V alpha 2 chain/FAA      | 10                    | 0.67         | 0.237   |
| TTR         | Transthyretin amyloidosis/DCM          | 2                     | 1.48         | 0.247   |
| SHOC2       | Soc-2 suppressor of clear homolog (C. elegans)/NS | 2                     | 0.32         | 0.252   |
| SCN4B       | Sodium channel, voltage-gated, type IV, beta/LQTS | 1                     | 0.10         | 0.262   |
| KCNE1       | Potassium voltage-gated channel, Ik-related family, member 1/LQTS | 1                     | 0.32         | 0.262   |
| PRKAG2      | Protein kinase, AMP-activated, gamma 2 noncatalytic subunit/DCM | 3                     | 0.52         | 0.277   |
| MYL3        | Myosin, light chain 3, alkali; ventricular, skeletal, slow/DCM | 1                     | 0.12         | 0.279   |
| SCN2B       | Sodium channel, voltage-gated, type II, beta subunit/BrS | 2                     | 0.34         | 0.279   |
| CACNA1C     | Calcium channel, voltage-dependent, I, alpha 1C subunit/LQTS | 11                    | 1.62         | 0.285   |
| TGFBR1      | Transforming growth factor, beta receptor 1/MFS | 1                     | 0.11         | 0.288   |
| ILK         | Integrin-linked kinase/DCM             | 1                     | 0.13         | 0.298   |
| FHL1        | Four and a half LIM domains 1/DCM      | 1                     | 0.13         | 0.298   |
| JPH2        | Junctophilin 2/DCM                      | 6                     | 1.52         | 0.298   |
| MYH7        | Myosin, heavy chain 7, cardiac muscle, beta/DCM | 4                     | 1.96         | 0.300   |
| LMFI        | Lipase maturation factor 1/FH           | 13                    | 1.21         | 0.304   |
| NOTCH1      | Notch 1/FAA                            | 24                    | 0.78         | 0.317   |
| SMAD4       | Small mothers against decapentaplegic (drosophila name), transcription factors/DCM | 1                     | 7.90         | 0.317   |
| ALMS1       | Centrosome- and basal body-associated protein/DCM | 44                   | 1.18         | 0.319   |
| CTF1        | Cardiotrophin 1/DCM                    | 1                     | 2.80         | 0.323   |
| GCKR        | Glucokinase regulatory protein/FH       | 7                     | 0.53         | 0.341   |
| SOS1        | Son of sevenless homolog 1 (Drosophila)/DCM | 4                     | 0.51         | 0.342   |
| RAF1        | v-raf-1 murine leukemia viral oncogene homolog 1/DCM | 1                     | 6.37         | 0.349   |
| Gene symbol | Gene name/associated cardiac syndrome* | Combined variants, n | Odds ratios+ | p value |
|-------------|----------------------------------------|----------------------|-------------|---------|
| LAMA4       | Laminin, alpha 4/DCM                  | 11                   | 1.39        | 0.358   |
| SCN5A       | Sodium channel, voltage-gated, type V, alpha subunit/ARVC | 15                   | 0.76        | 0.359   |
| SLC2A10     | Solute carrier family 2 (facilitated glucose transporter), member 10/FAA | 8                     | 0.87        | 0.360   |
| KRAS        | v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog/NS | 1                     | 6.33        | 0.363   |
| CSRP3       | Cysteine and glycine-rich protein 3 (cardiac LIM protein)/HCM | 1                     | 6.09        | 0.364   |
| PITPN11     | Protein tyrosine phosphatase, nonreceptor type 11/HCM | 1                     | 6.09        | 0.364   |
| FKRP        | Fukutin-like protein (FKRP)/DCM       | 6                     | 0.60        | 0.366   |
| KLF10       | Kruppel-like factor 10/HCM            | 6                     | 0.66        | 0.369   |
| FBN1        | Fibrillin 1/MFS                       | 15                   | 0.70        | 0.372   |
| CALR3       | Calreticulin 3/HCM                    | 4                     | 1.59        | 0.382   |
| LAMA2       | Laminin, alpha 2/HCM                 | 42                   | 0.89        | 0.386   |
| SGCD        | Sarcoglycan, delta (35 kDa dystrophin-associated glycoprotein)/HCM | 2                     | 1.56        | 0.388   |
| TMEM43      | Transmembrane protein 43/ARVC         | 4                     | 1.42        | 0.397   |
| COX15       | COX15 homolog, cytochrome c oxidase assembly protein (yeast)/HCM | 6                     | 0.60        | 0.415   |
| MYRN        | Myopalladin/DCM                      | 15                   | 1.14        | 0.433   |
| KCNA5       | Potassium voltage-gated channel subfamily a member 5/familial atrial fibrillation | 10                   | 1.35        | 0.433   |
| TMPO        | Thymopoietin/HCM                     | 13                   | 0.88        | 0.457   |
| SALL4       | Spalt-like transcription factor 4/congenital heart disease | 9                     | 0.72        | 0.461   |
| TGFBR2      | Transforming growth factor, beta 2/FAA | 3                     | 0.42        | 0.462   |
| PDLM3       | PDZ and LIM domain 3/HCM             | 5                     | 0.77        | 0.465   |
| SCN1B       | Sodium channel subunit beta-1/BrS     | 8                     | 1.60        | 0.473   |
| TRDN        | Triadin/CPVT                          | 11                   | 0.87        | 0.489   |
| CRL         | Cbl proto-oncogene, E3 ubiquitin protein ligase/NS | 3                     | 0.45        | 0.490   |
| KRT17       | Keratin 17/possible arrhythmias       | 2                     | 1.48        | 0.495   |
| SREBF2      | Sterol regulatory element-binding transcription factor 2/FH | 10                   | 1.21        | 0.513   |
| SGCG        | Sarcoglycan gamma/DCM                | 4                     | 1.19        | 0.529   |
| RAG3        | BCL2-associated athanogene 3/DCM     | 8                     | 1.15        | 0.533   |
| DOLK        | Dolichol kinase/DCM                  | 6                     | 1.66        | 0.538   |
| ABCG8       | ATP-binding cassette, subfamily G (WHITE), member 8/FH | 17                   | 0.86        | 0.538   |
| PRDM16      | PR domain containing 16/HCM          | 14                   | 1.29        | 0.558   |
| DISP        | Desmoplakin/ARVC                     | 23                   | 0.89        | 0.564   |
| RYR2        | Ryanodine receptor 2 (cardiac)/ARVC   | 15                   | 0.90        | 0.567   |
| CACNB2      | Calcium channel, voltage-dependent, beta 2 subunit/BrS | 8                     | 0.71        | 0.573   |
| KCNE2       | Potassium voltage-gated channel, Isk-related family, member 2/LQTS | 3                     | 0.68        | 0.573   |
| RBM20       | RNA-binding motif protein 20/DCM     | 16                   | 1.17        | 0.577   |
| AP0B        | Apolipoprotein B (including Ag(x) antigen)/FH | 56                   | 0.94        | 0.583   |
| EYA4        | EYA transcriptional coactivator and phosphatase 4/DCM | 5                     | 0.70        | 0.584   |
| MYO6        | Myosin VI/HCM                        | 8                     | 0.74        | 0.586   |
| AP0A5       | Apolipoprotein A5/FH                 | 3                     | 1.25        | 0.604   |
| FH1L2       | Four and a half LIM domains 2/HCM    | 3                     | 0.70        | 0.607   |
| GLA         | Galactosidase, alpha/HCM             | 4                     | 1.43        | 0.608   |
| TTN         | Titan/HCM                            | 437                  | 1.01        | 0.608   |
| CRELD1      | Cysteine rich with EGF-like domains 1/atrial septal defect | 4                     | 0.84        | 0.609   |
| AKAP9       | A kinase (PRKA) anchor protein (yotiao) 9/LQTS | 30                   | 0.91        | 0.617   |
| DTNA        | Dystrobrevin, alpha/LVNC            | 5                     | 1.42        | 0.619   |
| MRD1        | Mindbomb E3 ubiquitin protein ligase 1/LVNC | 3                     | 1.73        | 0.633   |
| NEXN        | Nexlin (F actin binding protein)/HCM | 7                     | 0.88        | 0.634   |
| TBX5        | T-box transcription factor 5/congenital heart disease and atrial fibrillation | 5                     | 0.76        | 0.644   |
| SDHA        | Succinate dehydrogenase complex flavoprotein subunit A/rare cardiomyopathy and pheochromocytoma | 13                   | 0.92        | 0.645   |
| CRYAB       | Crystallin, alpha B/HCM              | 2                     | 1.71        | 0.652   |
| GPIHBP1      | Glycosylphosphatidylinositol-anchored high-density lipoprotein-biding protein 1/FH | 3                     | 1.19        | 0.653   |
| DSC2        | Desmocollin 2/HCM                   | 11                   | 0.88        | 0.659   |
| KCNE3       | Potassium voltage-gated channel, Isk-related family, member 3/LQTS | 3                     | 1.66        | 0.661   |
| DPPE        | Dipeptidyl peptidase like 6/FH      | 11                   | 1.11        | 0.673   |
| COL5A1      | Collagen type V alpha 1 chain/Ehlers-Danlos and cardiac repair | 20                   | 0.89        | 0.677   |
| CREB3L3     | CAMP responsive element-binding protein 3 like 3/FH | 3                     | 1.62        | 0.680   |
We conducted a gene-level burden test to identify genes that are associated with SCD risk. Different from traditional individual variant analysis, the gene-level burden test aggregates minor alleles for the variants that are in the same gene to improve statistical power and reduce multiple testing [21]. At each gene, minor allele carrier status of each individual is assigned based on the variants within the gene. For example, those with at least 1 minor allele are assigned as a carrier, while those who do not have any minor alleles across all the variants in the gene are assigned as a noncarrier. This status is then tested for association with the disease status such as SCD case-control status. An odds ratio (OR) >1 would indicate that having minor alleles in the gene increases the risk for SCD, while an OR <1 would indicate that having minor alleles in the gene decreases the risk for SCD.

As the primary analysis, we conducted a gene-level burden test using the variants that passed filtering, as previously described. AFAn and EA samples were tested separately to reduce false discoveries due to population stratification that can arise when analyzing data across different racial groups.

| Gene symbol | Gene name/associated cardiac syndrome* | Combined variants, n | Odds ratios+ | p value |
|-------------|--------------------------------------|----------------------|-------------|---------|
| FKTN        | Fukutin/HCM                          | 6                    | 1.18        | 0.682   |
| HCN4        | Hyperpolarization activated cyclic nucleotide-gated potassium channel 4/BrS| 12                   | 0.88        | 0.688   |
| MYH6        | Myosin, heavy chain 6, cardiac muscle, alpha/HCM | 18                   | 0.92        | 0.716   |
| KCNH2       | Potassium voltage-gated channel, subfamily H (eag-related), member 2/LQTS | 8                     | 0.92        | 0.724   |
| SCO2        | SCO2 cytochrome c oxidase assembly protein/HCM | 1                     | 1.32        | 0.753   |
| DSG2        | Desmoglein 2/DCM                     | 17                   | 0.94        | 0.768   |
| DMD         | Dystrophin/DCM                       | 22                   | 0.96        | 0.774   |
| MYBPC3      | Myosin-binding protein C, cardiac/LVNC| 17                   | 1.07        | 0.779   |
| CETP        | Cholesteryl ester transfer protein, plasma/FH | 6                     | 0.93        | 0.782   |
| COL3A1      | Collagen, type III, alpha 1/FAA      | 5                     | 1.27        | 0.797   |
| NPPA        | Natriuretic peptide A/DCM           | 2                     | 0.91        | 0.810   |
| CAVIN4      | Caveola-associated protein 4/HCM     | 3                     | 1.20        | 0.818   |
| EFEMP2      | EGF-containing fibulin-like extracellular matrix protein 2/FAA | 3                     | 0.82        | 0.820   |
| TRIM63      | Tripartite motif containing 63, E3 ubiquitin protein ligase/HCM | 3                     | 0.83        | 0.824   |
| HADHA       | Hydroxyacyl-CoA dehydrogenase trifunctional multi-enzyme complex Subunit Alpha/DCM | 3                     | 0.88        | 0.825   |
| TCAP        | Titin-cap (telethonin)/DCM           | 4                     | 1.17        | 0.836   |
| JUP         | Junction plakoglobin/DCM             | 4                     | 1.09        | 0.844   |
| KCNQ1       | Potassium voltage-gated channel, KQT-like subfamily, member 1/LQTS | 7                     | 1.13        | 0.849   |
| ZIC3        | Zic family member 3/congenital heart disease | 2                     | 0.87        | 0.850   |
| BRAF        | v-raf murine sarcoma viral oncogene homolog B1/HCM | 4                     | 0.87        | 0.871   |
| TGFBR1      | Transforming growth factor, beta 3/ARVC | 4                     | 0.87        | 0.884   |
| TGFBR2      | Transforming growth factor, beta receptor II (70/80 kDa)/MFS | 4                     | 0.89        | 0.887   |
| KCNJ8       | Potassium inwardly rectifying channel, subfamily J, member 8/BrS | 1                     | 0.89        | 0.889   |
| LDLRAP1     | Low-density lipoprotein receptor adapter protein 1/FH | 2                     | 1.05        | 0.892   |
| ACTN2       | Actinin, alpha 2/HCM                | 10                    | 1.06        | 0.894   |
| LDB3        | LIM domain binding 3/DCM            | 10                    | 1.05        | 0.898   |
| GJA1        | Gap junction protein alpha 5 (connexin family)/atrial fibrillation | 2                     | 0.85        | 0.911   |
| SLC25A4     | Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4/HCM | 1                     | 0.85        | 0.911   |
| GAA         | Glucosidase, alpha; acid/HCM         | 11                    | 0.98        | 0.911   |
| RANGRF      | RAN guanine nucleotide release factor/BrS | 2                     | 0.86        | 0.914   |
| GDPD1L      | Glycerol-3-phosphate dehydrogenase 1-like/BrS | 2                     | 0.87        | 0.922   |
| PKP2        | Plakophilin 2/BrS                    | 16                    | 1.03        | 0.925   |
| MYLK2       | Myosin light chain kinase 2/HCM      | 9                     | 1.03        | 0.931   |
| SMAD3       | SMAD family member 3/FAA             | 1                     | 1.04        | 0.942   |
| LAMP2       | Lysosomal-associated membrane protein 2/HCAM | 2                     | 0.90        | 0.942   |
| RYR1        | Ryanodine receptor 1/malignant hyperthermia | 40                    | 0.99        | 0.945   |
| MAP2K2      | Mitogen-activated protein kinase 2/HCM | 1                     | 0.92        | 0.952   |
| FBN2        | Fibrillin 2/congenital heart disease | 28                    | 1.01        | 0.965   |
| TRPM4       | Transient receptor potential cation channel, subfamily M, member 4/BrS | 13                    | 1.00        | 0.994   |

*Indicates the inherited cardiac condition identified with abnormalities in this gene. ARVC, arrhythmogenic right ventricular cardiomyopathy; AVD, aortic valve disease; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; FAA, familial aortic aneurysm; FH, familial hypercholesterolemia; HCM, hypertrophic cardiomyopathy; LQTS, long QT syndrome; LVNC, left ventricular noncompaction; MFS, Marfan syndrome; NS, Noonan syndrome; RCM, restrictive cardiomyopathy; SQTS, short QT syndrome. Additional cardiac phenotypes are written out. + A positive hazard ratio indicates increased positive association with the disease, and a negative hazard ratio indicates protective against the disease.

Table 2 (continued)
lyzed together. Using individual population analysis results, meta-analysis was conducted to identify genes that show a consistent effect in both populations. There were 1,526 variants available for testing after filtering for AfAn and 1,624 variants for EA samples. We used RVTESTS [22] and RAREMETAL [23] to conduct a gene-level burden test and meta-analysis. After adjusting for 153 genes, our Bonferroni-corrected statistical significance threshold was $3.3 \times 10^{-04}$ ($0.05/153$).

As a secondary analysis, we conducted a burden test at the disease level based on the assigned categories from the initial study involved in the TruSight development [15]. Using the same aggregation strategy as we have used for the primary gene-level analysis, we determined the minor allele carrier status for each individual for the variants in the genes associated with each of the 15 ICC. For example, LQTS had 16 genes including 137 variants. For disease-burden testing, we assigned the minor allele carrier status for each individual based on the 137 variants that are in the 16 genes associated with LQTS. If a participant did not have any minor alleles across the 137 variants, then the participant was assigned as a non-carrier, while participants with at least 1 minor allele across the 137 variants were assigned as a carrier. Again, this carrier status was tested for association with the SCD status. Again, we tested the 2 (AfAn and EA) populations separately and then meta-analyzed to identify any consistent associations.

Last, we carried out a burden test at the gene functional level. Similar to the secondary burden test which was conducted at the disease level using the list of associated genes, we classified the genes into 11 functional categories based on GeneCards [24] reported gene function annotations.

To estimate the detectable ORs for gene-level burden testing in our dataset, we conducted a power calculation using GAS Power Calculator (http://csg.sph.umich.edu/abecasis/gas_power_calculator/index.html) [25]. We assumed a disease prevalence of 0.5% and combined a gene-level allele frequency of 5 and 10% (the proportion of samples with at least 1 minor allele for a gene). At 80% power, adjusted for multiple testing, this study was able to detect relative risks of 3.5 and 4.5 for a combined gene-level allele frequency of 10 and 5%, respectively. For genes with a carrier frequency of 1% (1% of the samples are minor allele carriers), a relative risk of 10 could be detected at 70% power.

**Results**

Baseline demographics and laboratory values are shown in Table 1. There were no statistical differences among the demographics and laboratory values between those with SCD and those without SCD. Compared with the full EVOLVE cohort, patients with SCD were older ($p = 1.7e^{-06}$), more likely to have diabetes ($p = 8.6e^{-05}$), and have a history of any arrhythmia ($p = 3.2e^{-4}$). Nearly identical

### Table 3. Disease burden test

| Disease          | Genes, n | Combined variants, n | Odds ratios | p value |
|------------------|----------|----------------------|-------------|---------|
| **Inherited arrhythmias** |          |                      |             |         |
| LQTS             | 16       | 137                  | 0.87        | 0.084   |
| SQTS             | 4        | 19                   | 1.11        | 0.576   |
| BrS              | 14       | 89                   | 1.03        | 0.796   |
| CPVT             | 6        | 29                   | 0.84        | 0.177   |
| **Cardiomyopathies** |        |                      |             |         |
| HCM              | 48       | 644                  | 1.01        | 0.644   |
| DCM              | 51       | 800                  | 1.01        | 0.672   |
| ARVC             | 12       | 556                  | 1.00        | 0.803   |
| RCM              | 9        | 33                   | 1.13        | 0.355   |
| LVNC             | 10       | 52                   | 1.14        | 0.397   |
| NS               | 9        | 16                   | 0.73        | 0.435   |
| **Aortopathies** |          |                      |             |         |
| MFS              | 4        | 41                   | 0.64        | 0.073   |
| LDS              | 3        | 20                   | 0.70        | 0.300   |
| FAA              | 12       | 92                   | 1.03        | 0.694   |
| AVD              | 3        | 51                   | 0.96        | 0.832   |
| FH               | 8        | 120                  | 0.93        | 0.361   |

LQTS, long QT syndrome; SQTS, short QT syndrome; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; ARVC, arrhythmogenic right ventricular cardiomyopathy; RCM, restrictive cardiomyopathy; LVNC, left ventricular noncompaction; NS, Noonan syndrome; MFS, Marfan syndrome; LDS, Loeys-Dietz syndrome; FAA, familial aortic aneurysm; AVD, aortic valve disease; FH, familial hypercholesterolemia.
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Table 4. Gene function burden test

| Gene function grouping               | Combined variants, n | Odds ratio | p value  |
|--------------------------------------|----------------------|------------|----------|
| Adaptor signal transduction          | 15                   | 1.20       | 0.395    |
| Development gene expression          | 133                  | 0.99       | 0.948    |
| Ion channel                          | 264                  | 0.94       | 0.278    |
| Lipid metabolism                     | 133                  | 0.93       | 0.346    |
| Metabolism                           | 53                   | 1.00       | 0.998    |
| Mitochondria                         | 25                   | 0.85       | 0.295    |
| Signaling RAS/MAPK                    | 18                   | 0.81       | 0.566    |
| Transport                            | 46                   | 1.00       | 0.994    |
| Vascular connective tissue           | 123                  | 0.91       | 0.411    |
| Cytoskeleton                         | 853                  | 1.01       | 0.530    |
| Signal transduction                  | 161                  | 0.92       | 0.274    |

Discussion/Conclusion

In the present study, we aimed to determine whether genes known to be associated with hereditary cardiovascular disease may be associated with adjudicated SCD events in patients with ESKD from the EVOLVE trial. No significant associations were observed, although our power was limited. Despite strong evidence of heritability with cardiac arrest based on CMS data [8], we did not see significant associations. A previous study in patients with ESKD [9] examined 24 genes in 47 patients and 38 with long QTc present on electrocardiogram characterized as abnormal before, after, or before and after dialysis. Two of the 5 patients with potassium channel defects (KCNH2 and KCNE1) subsequently died of SCD. These same 24 genes were examined in the present study, but we were unable to identify association between KCNH2 and KCNE1 and SCD in our samples (p = 0.72 and 0.26, respectively). To our knowledge, these are the only 2 studies to examine potential genetic risks in patients with ESKD and SCD. Our study of 126 patients with SCD, though much larger than Coll et al. [26], is still underpowered to detect rare variants. Thus, the overall results of this modest-sized study suggest that known genes associated with hereditary genetic diseases in the general population do not explain the high rates of SCD in patients undergoing dialysis. Our previous work did however find associations of SNPs in the ACE gene with decreased risk of SCD in EA and another SNP with increased risk of SCD in AfAn [10].

There are many risk factors for SCD in patients with ESKD. First, our study showed that compared to the overall EVOLVE cohort, patients with SCD were more likely to be older, have diabetes, and with a previous history of arrhythmia. Second, left ventricular hypertrophy, present in 80% of patients starting dialysis, increases the risk [27, 28]. Third, obstructive sleep apnea is common in patients undergoing dialysis [29] and is associated with a higher risk of SCD after adjusting for demographics (HR 3.28 [95% CI 1.12–9.57]) [29]. Fourth, 70% of patients starting dialysis have significant coronary artery calcification [30], which has been shown to predict SCD and Framingham risk factors in the general population [31]. Finally, disordered fluid and electrolyte fluxes with dialysis may still be causative, although we did not find any differences in serum electrolytes between those with and without SCD in this study, and Pun et al. [32], analyzing the entire cohort in the EVOLVE study, found no relationship between dialysate calcium concentration and cardiovascular events, including SCD.

Unfortunately, we still lack a complete understanding of the pathophysiology of SCD, making treatment and/or...
prevention difficult. In the general population, implantable defibrillators are the treatment of choice, especially with reduced ejection fraction. A meta-analysis demonstrated that implantable defibrillators reduced mortality in non-dialysis-requiring CKD but not in patients undergoing dialysis [33]. The ICD-2 trial confirmed a lack of efficacy in 188 patients with ESKD and an ejection fraction >35% [34]. The type of fatal arrhythmia in ESKD may be different from the general population with SCD. Five studies have utilized implantable loop records during and between dialysis sessions, with a follow-up from 6 to 21 months [33–37]. All of these studies noted a high rate of both atrial fibrillation and bradycardia, the latter affecting 25–30% of patients with only a minority of patients suffering from ventricular arrhythmias. Three studies found increased arrhythmias during the long (3-day) versus 2-day interval between hemodialysis sessions, suggesting the magnitude of volume retention (or accumulation of uremic toxins) may play a role [33, 36, 37]. In the general population, the treatment of nonatherosclerotic SCD similarly remains unclear [35], although genetic testing is suggested in patients with a family history of an early cardiac event. Based on the current study, we cannot recommend such an approach in patients with ESKD.

This study has several limitations, most importantly, sample size, although it remains the largest study to date. The modest sample size severely limited the statistical power of the study, only allowing identification of association with genes having large relative risks (RR > 3.5). The sample tested may not be generalizable to the hemodialysis population at large, as 70% of the patients were on dialysis for >2 years since all had moderate to severe hyperparathyroidism. It is conceivable that individuals with rare genetic variants may have died earlier after dialysis initiation. Despite these limitations, the study tested the most likely rare genetic variants and, importantly, utilized endpoints that were adjudicated by an independent committee.

In conclusion, we examined rare cardiovascular gene variants in a case-control study of prevalent patients on hemodialysis, matched for age, sex, diabetes, and duration of dialysis. None of the tested genes had different burden for rare function variants in patients who suffered from SCD compared to the matched controls. These data do not support genetic testing to stratify patients on dialysis for risk of SCD.

Statement of Ethics

This was a substudy of a clinical trial (EVOLVE, NCT00345839) using de-identified DNA samples and was considered “not subject to common rule” per our Institutional Review Board.

Conflicts of Interest Statement

Dr. Moe and Dr. Chertow served in the Academic Executive Committee and Publication Committee for the EVOLVE trial. Dr. Chertow has received funding from Amgen to conduct secondary analyses of the EVOLVE trial. Dr. Vatta receives salary and stocks from Invitae Corporation.

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Author Contributions

The project was conceived and funding obtained by S.M.M. and G.M.C. T.-H.S.-A., M.V., M.A., and L.W. conducted the analyses. All the authors worked together to interpret the results. T.-H.S.-A. and S.M.M. drafted the manuscript, and all the authors approved the final submission.

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