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The contribution of known familial cardiovascular disease genes to sudden cardiac death in patients undergoing hemodialysis

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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 to 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 Ancestry [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 to 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) to capture the genetic regions of interest. In all, the kit targets 572 kb that includes 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 gene 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.

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Author Contributions: The project was conceived and funding obtained by SMM and GMC. TS-A, MV, MA, LW conducted the analyses. All authors worked together to interpret the results. TS-A and SMM drafted the manuscript and all authors approved the final submission.

Discussion/Conclusions: We found no associations among 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.

Keywords

Cardiovascular; Sudden Cardiac Death; Arrhythmia; Dialysis; Kidney

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, or ESKD), cardiovascular manifestations change from one of predominant atherosclerotic disease to one of left ventricular hypertrophy and heart failure with preserved ejection fraction (HFpEF). 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 that CKD itself, and/or its attendant metabolic abnormalities, may be a risk factor for SCD. Importantly, a study of patients undergoing dialysis in the Fresenius Medical Care ESKD database identified pairs of genetically related family members who had suffered from a fatal cardiac arrest/arrhythmia, and phenotypically matched control pairs. The risk of cardiac arrest/arrhythmia was increased 1.88 (95% CI 1.25–2.84) for family members who did not cohabitate[8], suggesting a possible genetic cause of SCD.

A study of forty-seven patients with ESKD and prolonged QT interval found five genes associated with arrhythmias; in follow up, two patients died of SCD [9]. We previously reported that three 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], have 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 end points from the EVOLVE trial [12, 13].

MATERIALS AND METHODS

Participant Selection

The EVOLVE trial randomized 3883 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, non-fatal myocardial infarction, hospitalization for unstable angina, heart failure and peripheral vascular event. A post hoc analysis [4] found that 54% of all deaths in EVOLVE were due to cardiovascular disease, and of those, nearly one in four (352 patients) were 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 approved by Indiana University Institutional Review Board[14]. Of those 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 to 5 and greater than 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 non-compaction (LVNC), Noonan syndrome, Marfan syndrome (MFS), Loeys-Dietz syndrome (LDS), Familial aortic aneurysm, Aortic valve disease, and Familial Hypercholesterolemia), we used the TruSight Cardio Kit (Illumina, San Diego CA) to capture the coding regions of the reported genes. In all, the kit targets 572 kb that include the protein coding regions and 40 bp 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 Indiana University School of Medicine (IUSM) using MiSeq (Illumina, San Diego CA) following the manufacturer’s procedures.

Sequence data processing

We processed sequence data using the in-house NextGen sequence data pipeline that uses GATK4 (Genome Analysis Tool Kit version 4)[16] following the Best Practices [17] guideline. Briefly, reads were first mapped to human reference genome build 38 (hg38) using BWA [18]. We then sorted, de-duplicated, and re-calibrated base quality scores from mapped reads using GATK4. 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 single nucleotide polymorphisms (SNPs) and Insertion/Deletions (InDels). Due to the limited number of samples, as well as limited size of sequenced regions, we filtered variants based on the parameters (below) instead of the more typical variant quality score recalibration (VQSR) procedure in GATK. We required the following; a) Quality by Depth (QD) ≥ 2.0, b) Fisher Strand (FS) ≤ 60.0, c) Root Mean Square of the mapping quality of the reads across all samples (MQ) ≥ 40.0, d) Mapping quality rank sum test statistic (MQRankSum) ≥ −12.5, e) Read position rank sum test statistic (ReadPosRankSum) ≥ 8.0, and f) Strand Odds Ratio (SOR) ≤ 3.0 for SNPs in the vcf file. For the InDels we required the following; a) QD ≥ 2.0, b) FS ≤ 200.0, c) ReadPosRankSum ≥ 20.0, and d) SOR ≤ 3.0. After filtering, we annotated all variants that passed QC using ANNOVAR [19].

Variant selection for analysis

Variants passing QC as described above 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 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 greater than or equal to 5% based on reported population level frequencies from gnomAD Non-Finish European and African samples. After filtering for predicted function (amino acid coding changing) and population based minor allele frequencies (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 entire EVOLVE cohort after removing those SCD cases) by Chi Squared test for categorical items and two sample t-test for continuous items. After post hoc adjustment based on the 28 comparisons required a p-value of 0.0018 to indicate statistically significant difference in the two groups.

We conducted gene-level burden test to identify genes that are associated with SCD risk. Different from traditional individual variant analysis, 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 one 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 non-carrier. This status is then tested for association with disease status such as SCD case/control status. Odds ratio greater than 1 would indicate that having minor alleles in the gene increases risk for SCD while an odds ratio less than 1 would indicate that having minor alleles in the gene decreases risk for SCD.

As the primary analysis, we conducted 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 analyzed together. Using individual population analyses results, meta-analysis was conducted to identify genes that show consistent effect in both populations. There were 1,526 variants available for testing after filtering for AfAa samples and 1,624 variants for EA samples. We used RVTESTS [22] and RAREMETAL [23] to conduct gene-level burden test and meta-analysis. After adjusting for 153 genes, our Bonferroni corrected statistical significance threshold was $3.3X10^{-04}$ (0.05/153).

As a secondary analysis, we conducted a burden test at disease level based on the assigned categories from the initial study involved in the TruSight development[15].. Using the same aggregation strategy as we've 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, Long QT syndrome 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 that are associated with Long QT syndrome. 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 one minor allele across the 137 variants were assigned as carrier. Again, this carrier status was tested for association with SCD status. Again, we tested the two (AfAn and EA) population separately then meta-analyzed to identify any consistent associations. Lastly, we carried out burden test at gene functional level. Similar to the secondary burden test which was conducted at 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 odds ratios 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 gene-level allele frequency of 5 and 10% (the proportion of samples with at least one 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 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 was 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 differences were observed when those tested in the current study were removed from the EVOLVE cohort. The burden test groups rare variants into a single variant for analyses as detailed above. Table 2 lists the gene name, its disease association, and number of variants examined in each gene. The gene with the largest number of variants is TTN (titin), but that is also the largest gene. After adjusting for the length of captured region, the largest number of variants were found in *APOC2*, followed by *KCNE3*. Eleven genes showed a significant association between gene-burden and SCD

($p < 0.05$), but were not statistically significant association after multiple testing correction. All gene-level burden test results are shown in Table 2, and the specific references for each gene can be found in the supplemental table of [15]. To determine if genes relating to one disease (inherited cardiac condition as denoted by the Tru-Site kit, for example dilated versus hypertrophic cardiomyopathy) were more commonly associated with patients suffering SCD versus controls, we grouped genes by their associated disease and performed a burden test. (Table 3). We did not observe any associations with p-value less than 0.05 in all 15 ICCs tested (Table 3). Lastly, to determine if a similar mechanism may be pathogenic in patients with SCD versus controls (for example potassium/sodium channel abnormalities), we grouped genes by their pathophysiology/function as noted by the Tru-Site kit. (Table 4). The burden testing at gene function level also did not yield any associations with p-value less than 0.05 in all 11 functional categories tested with SCD. Overall, we observed no statistically significant associations between the rare variant burden at gene, associated disease, and gene function levels compared to SCD status in our EVOLVE trial.

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, 38 with long QTc present on electrocardiogram characterized as abnormal before, after, or before and after dialysis. Two of the five 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 two studies to examine potential genetic risks in patients with ESKD and SCD. Our study of 126 patients with SCD, while 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 previous history of arrhythmia. Second, left ventricular hypertrophy, present in 80 percent of patients starting dialysis, increases the risk[27, 28]. Third, obstructive sleep apnea is common in patients undergoing dialysis[29], and is associated with 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 as well as 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, analyzing the entire cohort in the EVOLVE study, found no relationship between dialysate calcium concentration and cardiovascular events, including SCD[32].

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 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 more than two 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 end points 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 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.

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Statement of Ethics: that 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.

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

REFERENCES:

1. Baigent C, Landray M, Leaper C, Altmann P, Armitage J, Baxter A, et al.First United Kingdom Heart and Renal Protection (UK-HARP-I) study: biochemical efficacy and safety of simvastatin and safety of low-dose aspirin in chronic kidney disease. Am J Kidney Dis. 20053;45(3):473–84. [PubMed: 15754269]
2. Landray M, Baigent C, Leaper C, Adu D, Altmann P, Armitage J, et al.The second United Kingdom Heart and Renal Protection (UK-HARP-II) Study: a randomized controlled study of the biochemical safety and efficacy of adding ezetimibe to simvastatin as initial therapy among patients with CKD. Am J Kidney Dis. 20063;47(3):385–95. [PubMed: 16490616]
3. Herzog CA, Asinger RW, Berger AK, Charytan DM, Diez J, Hart RG, et al.Cardiovascular disease in chronic kidney disease. A clinical update from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int. 20119;80(6):572–86. [PubMed: 21750584]
4. Wheeler DC, London GM, Parfrey PS, Block GA, Correa-Rotter R, Dehmel B, et al.Effects of cinacalcet on atherosclerotic and nonatherosclerotic cardiovascular events in patients receiving hemodialysis: the EValuation Of Cinacalcet HCl Therapy to Lower CardioVascular Events (EVOLVE) trial. J Am Heart Assoc. 20141117;3(6):e001363.
5. Herzog CA. Can we prevent sudden cardiac death in dialysis patients?Clin J Am Soc Nephrol. 20075;2(3):410–2. [PubMed: 17699443]
6. Alqahtani F, Almustafa A, Shah K, Akram Y, Abbasi D, Rattan R, et al.Sudden cardiac arrest in end-stage renal disease patients on dialysis: A nationwide study. Pacing Clin Electrophysiol. 201811;41(11):1467–75. [PubMed: 30225901]
7. Pun PH. The interplay between CKD, sudden cardiac death, and ventricular arrhythmias. Adv Chronic Kidney Dis. 201411;21(6):480–8. [PubMed: 25443573]
8. Chan KE, Newton-Cheh C, Gusella JF, Maddux FW. Heritability of Risk for Sudden Cardiac Arrest in ESRD. J Am Soc Nephrol. 201511;26(11):2815–20. [PubMed: 25882830]
9. Coll M, Ferrer-Costa C, Pich S, Allegue C, Rodrigo E, Fernandez-Fresnedo G, et al.Role of genetic and electrolyte abnormalities in prolonged QTc interval and sudden cardiac death in end-stage renal disease patients. PLoS One. 2018;13(7):e0200756.
10. Moe SM, Long J, Schwantes-An TL, Decker BS, Wetherill L, Edenberg HJ, et al.Angiotensin-related genetic determinants of cardiovascular disease in patients undergoing hemodialysis. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 201872.
11. Bezzina CR, Lahrouchi N, Priori SG. Genetics of sudden cardiac death. Circ Res. 201565;116(12):1919–36. [PubMed: 26044248]
12. Chertow GM, Correa-Rotter R, Block GA, Drueke TB, Floege J, Goodman WG, et al.Baseline characteristics of subjects enrolled in the Evaluation of Cinacalcet HCl Therapy to Lower Cardiovascular Events (EVOLVE) trial. Nephrol Dial Transplant. 20127;27(7):2872–9. [PubMed: 22529163]
13. Investigators ET, Chertow GM, Block GA, Correa-Rotter R, Drueke TB, Floege J, et al.Effect of cinacalcet on cardiovascular disease in patients undergoing dialysis. N Engl J Med. 20121227;367(26):2482–94. [PubMed: 23121374]
14. Moe SM, Wetherill L, Decker BS, Lai D, Abdalla S, Long J, et al.Calcium-Sensing Receptor Genotype and Response to Cinacalcet in Patients Undergoing Hemodialysis. Clin J Am Soc Nephrol. 201777;12(7):1128–38. [PubMed: 28630081]
15. Pua CJ, Bhalshankar J, Miao K, Walsh R, John S, Lim SQ, et al.Development of a Comprehensive Sequencing Assay for Inherited Cardiac Condition Genes. J Cardiovasc Transl Res. 20162;9(1):3–11. [PubMed: 26888179]
16. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al.The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome research. 20109;20(9):1297–303. [PubMed: 20644199]
17. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, et al.From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr Protoc Bioinformatics. 2013;43:1110 1–33. [PubMed: 25431634]

18. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics (Oxford, England). 2009715;25(14):1754–60.
19. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 20109;38(16):e164. [PubMed: 20601685]
20. Karczewski KJ FL, Tiac G, Cummings BB, Alfoldi J, Wang Q, Collins RL, et al.Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. biorxiv. 2019.
21. Povysil G, Petrovski S, Hostyk J, Aggarwal V, Allen AS, Goldstein DB. Rare-variant collapsing analyses for complex traits: guidelines and applications. Nature Reviews Genetics. 2019 2019/12/01;20(12):747–59.
22. Zhan X, Hu Y, Li B, Abecasis GR, Liu DJ. RVTESTS: an efficient and comprehensive tool for rare variant association analysis using sequence data. Bioinformatics (Oxford, England). 201651;32(9):1423–6.
23. Feng S, Liu D, Zhan X, Wing MK, Abecasis GR. RAREMETAL: fast and powerful meta-analysis for rare variants. Bioinformatics. 201410;30(19):2828–9. [PubMed: 24894501]
24. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al.The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. Curr Protoc Bioinformatics. 2016620;54:1 30 1–1 30 33.
25. Skol AD, Scott LJ, Abecasis GR, Boehnke M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nature genetics. 20062;38(2):209–13. [PubMed: 16415888]
26. Coll B, Betriu A, Martinez-Alonso M, Borras M, Craver L, Amoedo ML, et al.Cardiovascular risk factors underestimate atherosclerotic burden in chronic kidney disease: usefulness of non-invasive tests in cardiovascular assessment. Nephrol Dial Transplant. 2010317.
27. Spirito P, Maron BJ. Relation between extent of left ventricular hypertrophy and occurrence of sudden cardiac death in hypertrophic cardiomyopathy. J Am Coll Cardiol. 19906;15(7):1521–6. [PubMed: 2140576]
28. Zoccali C, Benedetto FA, Mallamaci F, Tripepi G, Giacone G, Stancanelli B, et al.Left ventricular mass monitoring in the follow-up of dialysis patients: prognostic value of left ventricular hypertrophy progression. Kidney Int. 20044;65(4):1492–8. [PubMed: 15086493]
29. Kerns ES, Kim ED, Meoni LA, Sozio SM, Jaar BG, Estrella MM, et al.Obstructive Sleep Apnea Increases Sudden Cardiac Death in Incident Hemodialysis Patients. Am J Nephrol. 2018;48(2):147–56. [PubMed: 30110675]
30. Block GA, Spiegel DM, Ehrlich J, Mehta R, Lindbergh J, Dreisbach A, et al.Effects of sevelamer and calcium on coronary artery calcification in patients new to hemodialysis. Kidney Int. 200510;68(4):1815–24. [PubMed: 16164659]
31. Taylor AJ, Burke AP, O'Malley PG, Farb A, Malcom GT, Smialek J, et al.A comparison of the Framingham risk index, coronary artery calcification, and culprit plaque morphology in sudden cardiac death. Circulation. 2000321;101(11):1243–8. [PubMed: 10725282]
32. Pun PH, Abdalla S, Block GA, Chertow GM, Correa-Rotter R, Dehmel B, et al.Cinacalcet, dialysate calcium concentration, and cardiovascular events in the EVOLVE trial. Hemodial Int. 20167;20(3):421–31. [PubMed: 26564024]
33. Fu L, Zhou Q, Zhu W, Lin H, Ding Y, Shen Y, et al.Do Implantable Cardioverter Defibrillators Reduce Mortality in Patients With Chronic Kidney Disease at All Stages?Int Heart J. 2017531;58(3):371–77. [PubMed: 28539571]
34. Jukema JW, Timal RJ, Rotmans JI, Hensen LCR, Buiten MS, de Bie MK, et al.Prophylactic Use of Implantable Cardioverter-Defibrillators in the Prevention of Sudden Cardiac Death in Dialysis Patients. Circulation. 201964;139(23):2628–38. [PubMed: 30882234]
35. Kadakia RS, Link MS, Dominic P, Morin DP. Sudden cardiac death in nonischemic cardiomyopathy. Prog Cardiovasc Dis. 2019May-Jun;62(3):235–41. [PubMed: 31075279]

Table 1:

Baseline Demographics and Laboratory Values

	SCD cases n = 126	SCD controls n = 107	Evolve cohort n = 3883
Cinacalcet use, n (%)	60 (48)	52 (49)	1948 (50)
Age, years, mean (SD)	59.7 (11.5)	59.6 (11.8)	54.4 (14.4)[+]
Sex (female), n (%)	51 (40)	44 (41)	1578 (41)
Ethnicity (self-reported), n (%)			
European Ancestry	89 (71)	73 (68)	2240 (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)	1098 (28)
2 to < 5 years	45 (36)	37 (35)	1285 (33)
≥ 5 years	45 (36)	39 (36)	1499 (39)
Current or previous smoker, n (%)	58 (46)	53 (50)	1696 (44)
Abnormal baseline ECG, n (%)	92 (73)	70 (65)	2263 (58)
Diabetes (Type 1 or Type 2), n (%)	64 (51)	52 (49)	1302 (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)	3577 (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			
Calcium-based phosphate binders, n (%)	70 (56)	49 (46)	2062 (53)
Baseline vitamin D, n (%)	75 (60)	78 (73)	2310 (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 (356,1498)	597 (368,1281)	693 (363,1694)
FGF23, median (p10,p90), pg/mL	4380 (412,23914)	4465 (600,15095)	5590 (580,19528)
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 vs. entire EVOLVE cohort.

Table 2:

Gene Level Burden Tests

Gene Name	Gene name/associated cardiac syndrome*	Number of Combined Variants	Odd Ratios	p-value
APOA4	apolipoprotein A4/FH	8	0.40	0.006
CASQ2	calsequestrin 2 (cardiac muscle)/CPVT	2	0.10	0.015
APOE	apolipoprotein E/FH	2	0.12	0.017
ABCC9	ATP-binding cassette, sub-family C (CFTR/MRP), member 9/BrS	3	4.51	0.020
LDLR	low density lipoprotein receptor/FH	12	0.59	0.024
SNTA1	syntrophin, alpha 1 (dystrophin-associated protein A1, 59kDa, 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/HCM	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 (αB-crystallin)/DCM	2	6.08	0.111
LPL	lipoprotein lipase/FH	7	0.46	0.114
SGCB	sarcoglycan, beta (43kDa 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
LTBP2	latent transforming growth factor beta binding protein 2/MFS	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
APOC2	apoliprotein C2/FH	6	3.10	0.144
NKX2–5	NK2 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	2	5.91	0.203
TNNT2	troponin T type 2 (cardiac)/DCM	3	1.46	0.208
FXN	frataxin/HCM	3	0.54	0.228
VCL	vinculin/HCM	4	0.29	0.235

Gene Name	Gene name/associated cardiac syndrome*	Number of Combined Variants	Odd Ratios	p-value
COL5A2	collagen type V alpha 2 chain/FAA	10	0.67	0.237
TTR	Transthyretin amyloidosis/HCM	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, Isk-related family, member 1/LQTS	1	0.32	0.262
PRKAG2	protein kinase, AMP-activated, gamma 2 non-catalytic subunit/HCM	3	0.52	0.277
MYL3	myosin, light chain 3, alkali; ventricular, skeletal, slow/RCM	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, L type, 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/HCM	1	0.13	0.298
JPH2	junctophilin 2/HCM	6	1.52	0.298
MYH7	myosin, heavy chain 7, cardiac muscle, beta/DCM	4	1.96	0.300
LMF1	Lipase Maturation Factor 1/FH	13	1.21	0.304
NOTCH1	Notch 1/FAA	24	0.78	0.317
SMAD4	Small mothers against decapentaplegic (drospholia name), transcription factors/HCM	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)/HCM	4	0.51	0.342
RAF1	v-raf-1 murine leukemia viral oncogene homolog 1/HCM	1	6.37	0.349
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
PTPN11	protein tyrosine phosphatase, non-receptor type 11/HCM	1	6.09	0.364
FKRP	Fukutin-Related 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 (35kDa 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
MYPN	Myopalladin/DCM	15	1.14	0.433
KCNA5	potassium Voltage-Gated Channel Subfamily A Member 5/ familial atrial fibrillation	10	1.35	0.433

Gene Name	Gene name/associated cardiac syndrome*	Number of Combined Variants	Odd Ratios	p-value
TMPO	Thymopoietin/HCM	13	0.88	0.457
SALL4	spalt like transcription factor 4/congenital heart disease	9	0.72	0.461
TGFB2	transforming growth factor, beta 2/FAA	3	0.42	0.462
PDLIM3	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
CBL	Cbl proto-oncogene, E3 ubiquitin protein ligase/NS	3	0.45	0.490
KRT17	keratin 17/possible arrythmias	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
BAG3	BCL2-associated athanogene 3/DCM	8	1.15	0.533
DOLK	dolichol kinase/DCM	6	1.66	0.538
ABCG8	ATP-binding cassette, sub-family G (WHITE), member 8/FH	17	0.86	0.538
PRDM16	PR domain containing 16/HCM	14	1.29	0.558
DSP	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
APOB	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
APOA5	apolipoprotein A5/FH	3	1.25	0.604
FHL2	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
MIB1	mindbomb E3 ubiquitin protein ligase 1/LVNC	3	1.73	0.633
NEXN	nexilin (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 binding 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
DPP6	dipeptidyl peptidase like 6/FH	11	1.11	0.673

Gene Name	Gene name/associated cardiac syndrome*	Number of Combined Variants	Odd Ratios	p-value
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
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	Caveolae 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 Multienzyme 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
TGFB3	transforming growth factor, beta 3/ARVC	4	0.87	0.884
TGFBR2	transforming growth factor, beta receptor II (70/80kDa)/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 adaptor 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
GJA5	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
GPD1L	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/HCM	2	0.90	0.942

Gene Name	Gene name/associated cardiac syndrome*	Number of Combined Variants	Odd Ratios	p-value
RYR1	Ryanodine receptor 1/malignant hyperthermia	40	0.99	0.945
MAP2K2	mitogen-activated protein kinase 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 (ICC) identified with abnormalities in this gene: 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 non-compaction; NS = Noonan syndrome; MFS = Marfan syndrome; LDS = Loeys-Dietz syndrome; FAA= familial aortic aneurysm; AVD = aortic valve disease; FH = familial hypercholesterolemia. 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 3:

Disease Burden Test

Disease	Number of Genes	Number of Combined Variants	Odd Ratios	PVALUE
Inherited Arrhythmias				
Long QT syndrome	16	137	0.87	0.084
Short QT syndrome	4	19	1.11	0.576
Brugada syndrome	14	89	1.03	0.796
Catecholaminergic polymorphic ventricular tachycardia	6	29	0.84	0.177
Cardiomyopathies				
Hypertrophic cardiomyopathy	48	644	1.01	0.644
Dilated cardiomyopathy	51	800	1.01	0.672
Arrhythmogenic ventricular cardiomyopathy	12	556	1.00	0.803
Restrictive cardiomyopathy	9	33	1.13	0.355
Left ventricular non-compaction	10	52	1.14	0.397
Noonan syndrome	9	16	0.73	0.435
Aortopathies				
Marfan syndrome	4	41	0.64	0.073
Loeys-Dietz syndrome	3	20	0.70	0.300
Familial aortic anuerysm	12	92	1.03	0.694
Aortic valve disease	3	51	0.96	0.832
Familial Hypercholesterolaemia	8	120	0.93	0.361

Table 4:

Gene Function Burden Test

Gene Function Grouping	Number of Combined Variants	Odds Ratio	PVALUE
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