

# Rare Genetic Variants Associated With Sudden Cardiac Death in Adults

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## ABSTRACT

**BACKGROUND** Sudden cardiac death occurs in ~220,000 U.S. adults annually, the majority of whom have no prior symptoms or cardiovascular diagnosis. Rare pathogenic DNA variants in any of 49 genes can pre-dispose to 4 important causes of sudden cardiac death: cardiomyopathy, coronary artery disease, inherited arrhythmia syndrome, and aortopathy or aortic dissection.

**OBJECTIVES** This study assessed the prevalence of rare pathogenic variants in sudden cardiac death cases versus controls, and the prevalence and clinical importance of such mutations in an asymptomatic adult population.

**METHODS** The authors performed whole-exome sequencing in a case-control cohort of 600 adult-onset sudden cardiac death cases and 600 matched controls from 106,098 participants of 6 prospective cohort studies. Observed DNA sequence variants in any of 49 genes with known association to cardiovascular disease were classified as pathogenic or likely pathogenic by a clinical laboratory geneticist blinded to case status. In an independent population of 4,525 asymptomatic adult participants of a prospective cohort study, the authors performed whole-genome sequencing and determined the prevalence of pathogenic or likely pathogenic variants and prospective association with cardiovascular death.

**RESULTS** Among the 1,200 sudden cardiac death cases and controls, the authors identified 5,178 genetic variants and classified 14 as pathogenic or likely pathogenic. These 14 variants were present in 15 individuals, all of whom had experienced sudden cardiac death—corresponding to a pathogenic variant prevalence of 2.5% in cases and 0% in controls ( $p < 0.0001$ ). Among the 4,525 participants of the prospective cohort study, 41 (0.9%) carried a pathogenic or likely pathogenic variant and these individuals had 3.24-fold higher risk of cardiovascular death over a median follow-up of 14.3 years ( $p = 0.02$ ).

**CONCLUSIONS** Gene sequencing identifies a pathogenic or likely pathogenic variant in a small but potentially important subset of adults experiencing sudden cardiac death; these variants are present in ~1% of asymptomatic adults. (J Am Coll Cardiol 2019;■:■-■) © 2019 by the American College of Cardiology Foundation.

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**ABBREVIATIONS AND ACRONYMS****ACMG** = American College of Medical Genetics and Genomics**AMP** = Association of Molecular Pathology**CHF** = congestive heart failure**MESA** = Multi-Ethnic Study of Atherosclerosis

**A** primary goal of genomic medicine is to identify asymptomatic individuals at high inborn risk for a serious health consequence to enable targeted screening or prevention (1). This approach is particularly appealing for sudden cardiac death, a heritable and irreversible outcome that afflicts approximately 220,000 U.S. adults annually and often has a devastating impact on the surviving family and the community (2).

Recent advances in human genetics have identified 49 genes that harbor pathogenic variants predisposing to any of 4 important causes of sudden cardiac death—cardiomyopathy, coronary artery disease, inherited arrhythmia syndrome, and aortopathy or aortic dissection (3–5). If identified, risk stratification of pre-symptomatic individuals harboring such variants can be performed using tests already available in clinical practice and, in cases where high-risk features are recognized, evidence-based therapies to attenuate risk for each of the 4 conditions have been demonstrated (6–10).

A prior study conducted in the Dutch population assessed the prevalence of 6 pre-specified genetic

mutations using a genotyping approach, noting a prevalence of 1.1% in sudden cardiac death cases versus 0.4% in controls (11). This result suggests potential utility for gene sequencing studies, which enable ascertainment of all variants affecting the coding sequence of a given gene.

Previous gene sequencing studies have focused primarily on children and young adults who experienced sudden cardiac death unexplained by autopsy; these studies have suggested a rare predisposing genetic variant in 13% to 28% of afflicted individuals (12–15). However, the vast majority of sudden cardiac death occurs in middle-aged or elderly adults, most of whom do not undergo autopsy (2,16). Moreover, most prior studies have relied on retrospective case referral that can lead to ascertainment bias (12,14,15), implemented variable gene sequencing approaches (13,15), and did not assess the potential pathogenicity of observed variants using current clinical standards (12–14,17,18). Most importantly, all have lacked a control group subjected to identical genetic sequencing and variant classification (12–15).

We set out to overcome these previous limitations by using a matched control group, systematic gene sequencing, and blinded clinical-grade classification

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of variant pathogenicity. First, we first determined the extent to which pathogenic variants are enriched in adult-onset sudden cardiac death cases as compared to matched controls; and second, we quantified the prevalence and prognostic importance of such variants in a multiethnic population of asymptomatic middle-aged adults.

## METHODS

**STUDY POPULATIONS.** We assembled a nested case-control cohort for sudden cardiac death based on 6 previously described prospective studies. These studies included 4 randomized clinical trials—Physician's Health Study I and II (19,20), Women's Health Study (21), and Women's Antioxidant Cardiovascular Study (22)—and 2 prospective cohort studies, the Health Professionals Follow-up Study (23) and Nurses' Health Study (24). Together, these cohorts included a total of 38,975 men and 67,093 women with stored blood samples. Additional details on enrollment criteria and study design are available in [Online Table 1](#). For each case, a control was selected that matched as closely as possible based on study cohort, age at blood sample ( $\pm 1$  year), race/ethnicity, smoking status (current, never, or past), and presence or absence of atherosclerotic cardiovascular disease (myocardial infarction, coronary revascularization, angina, or stroke) at time of blood sample collection.

A multiethnic prospective cohort study of adults was derived from the Multi-Ethnic Study of Atherosclerosis (MESA), which enrolled individuals free of known cardiovascular disease or symptoms in the United States between 2000 and 2002 (25).

**SUDDEN CARDIAC DEATH ASCERTAINMENT (NESTED CASE-CONTROL STUDY).** All 6 prospective studies used similar methods to document the timing and mechanism of cardiovascular deaths as definite or probable sudden cardiac death as previously described (26)—including collection and review of medical records (inclusive of emergency medical services, emergency room, hospital, and autopsy reports as available) and standardized interviews with next-of-kin or witnesses to obtain detailed descriptions of the circumstances surrounding the death. Additional details are provided in the [Online Appendix](#).

**INCIDENT CARDIOVASCULAR DEATH AND INTERMEDIATE PHENOTYPE ASCERTAINMENT IN MESA.** Within the MESA study, incident cardiovascular deaths were adjudicated based on review of death certificates, hospitalization records, and next-of-kin interviews. Death adjudication was performed by an independent endpoints committee without access to

genetic data. Intermediate cardiovascular phenotypes, including lipid, electrocardiographic, and imaging phenotypes were ascertained by MESA core lab investigators (additional details available in the [Online Appendix](#)). Clinical hypercholesterolemia was defined as measured low-density lipoprotein (LDL)-cholesterol  $\geq 130$  mg/dl or use of statin medications (27). Prolonged corrected QT interval was defined as  $\geq 450$  ms in men or  $\geq 460$  ms in women (28). Left ventricular ejection fraction values were considered reduced if  $<52\%$  in men or  $<54\%$  in women and hyperdynamic if  $>72\%$  in men or  $>74\%$  in women (29).

**GENE SEQUENCING.** Whole-exome sequencing was performed on 1,200 samples of the sudden cardiac death nested case-control study and whole-genome sequencing was performed on the 4,525 MESA study participants at the Broad Institute of MIT and Harvard (Cambridge, Massachusetts). Additional details of gene sequencing methods are available in the [Online Appendix](#).

**FILTERING AND CLASSIFICATION OF VARIANTS.** We searched for pathogenic or likely pathogenic variants in 49 prespecified genes with known relevance to sudden cardiac death-related conditions cardiomyopathy (14 genes), coronary artery disease (3 genes), arrhythmia syndromes (24 genes), or aortopathy/aortic dissection (8 genes)—listed in [Online Table 2](#). We derived this list of 49 genes by extracting those related to cardiovascular disease in which pathogenic variants were deemed clinically actionable by the American College of Medical Genetics and Genomics (ACMG) or the Geisinger Health System, and additionally include the gene encoding titin (TTN), in which inactivating mutations are a leading cause of inherited cardiomyopathy (3–5).

Analysis was restricted to the protein-coding regions and canonical splice sites for each of the genes. We used bioinformatic filtering to first narrow the list of observed variants to candidate variants: predicted loss-of-function variants—those that lead to a premature stop codon (nonsense), disrupt canonical splice consensus sequence, exon splicing, or cause an inactivating frameshift (30); variants previously annotated as pathogenic or likely pathogenic in the ClinVar genetics database (31); and rare missense variants—those present with an allele frequency  $< 0.0001$  in each racial subpopulation of the gnomAD Genome Aggregation Database, a publicly available population allele frequency database of 123,136 exomes (30).

Candidate variants were then further narrowed to those meeting ACMG/Association of Molecular

**TABLE 1** Characteristics of Sudden Cardiac Death Cases and Controls

	Sudden Cardiac Death Cases (n = 600)	Matched Controls (n = 600)
Age at time of blood sample, yrs	64 ± 9	64 ± 9
Age at sudden death, yrs	72 ± 9	N/A
Male	395 (66)	395 (66)
White	581 (97)	585 (98)
ASCVD at time of blood sample	145 (24)	145 (24)
ASCVD at time of sudden death	232 (39)	N/A
Smoking status		
Never	225 (38)	230 (38)
Former	275 (46)	283 (47)
Current	94 (16)	85 (14)
Study cohort		
Physicians Health Study I	156	156
Physicians Health Study II	112	112
Health Professionals Follow-up Study	127	127
Nurses' Health Study	126	126
Women's Antioxidant Cardiovascular Study	41	41
Women's Health Study	38	38

Values are mean ± SD, n (%), or n.

ASCVD = atherosclerotic cardiovascular disease; N/A = not applicable.

Pathology (AMP) pathogenic or likely pathogenic criteria by an American Board of Genetics and Genomics-certified clinical laboratory geneticist within the Partners HealthCare Laboratory for Molecular Medicine who was blinded to case versus control status or any other phenotype information (17,18).

**STATISTICAL ANALYSIS.** Comparison of the number of observed genetic variants per individual in sudden cardiac death cases versus controls was performed with an unpaired Student's *t*-test. The 95% confidence interval (CI) for pathogenic variant prevalence was assessed using a binomial test. Association of pathogenic mutation status with sudden cardiac death case status was assessed using the Fisher exact test. The relationship of pathogenic variant status to incident cardiovascular death was determined using a Cox proportional hazards model adjusted for age, sex, and race. Statistical analyses were performed with the use of R software, version 3.4 (R Project for Statistical Computing).

## RESULTS

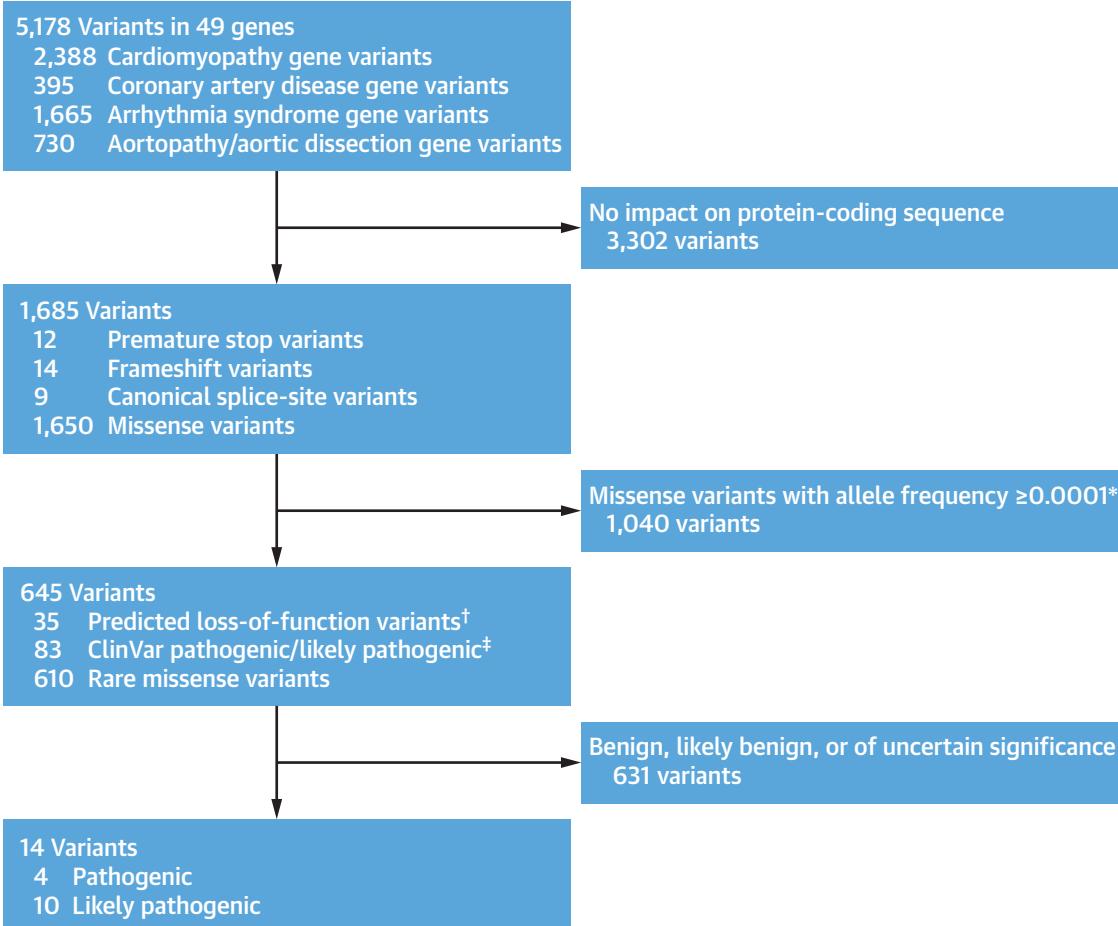
We performed whole-exome sequencing of 600 adult-onset sudden cardiac death cases and 600 matched controls. Mean age at time of blood draw was 64 years and 66% were male. Among cases, mean age at sudden cardiac death was 72 years (range, 48–98 years) (Table 1). Genetic background, as quantified by principal components of ancestry, was similar between cases and controls (Online Figure 1).

Among the 600 sudden cardiac death cases, 145 (24%) had been diagnosed with atherosclerotic cardiovascular disease at time of study enrollment and an additional 87 (15%) were diagnosed before sudden cardiac death. Chart review suggested a diagnosis of congestive heart failure (CHF) or cardiomyopathy before death in an additional 36 individuals (6%). However, the majority (n = 332; 55%) had not been diagnosed with atherosclerotic cardiovascular disease or CHF at the time they experienced sudden cardiac death.

Analysis of the 49 sudden cardiac death-related genes from whole-exome sequencing data identified a total of 5,178 genetic variants present in at least 1 of these 1,200 individuals. Synonymous DNA sequence variants—single base pair changes that have no impact on the amino acid sequence of the protein—are often used as a negative control in genetic case-control studies. A total of 1,096 of the identified 5,178 genetic variants were annotated as synonymous variants (Online Table 3). Consistent with expectations, the average number of synonymous variants in these 49 genes did not differ between cases and controls, with a mean 38.4 versus 38.3 variants per person, respectively (*p* = 0.99).

We next used bioinformatics filters to identify those variants that were predicted to cause loss of function, were extremely rare missense variants, or were previously annotated as likely to be pathogenic in the ClinVar genetics database. This filtering narrowed the list to 645 variants.

A clinical laboratory geneticist who was blinded to case/control status evaluated these 645 variants according to ACMG/AMP criteria (Figure 1)—631 were classified as benign, likely benign, or variants of uncertain significance. Cases and controls carried a similar number of these variants, with a mean 0.79 versus 0.75 variants per person, respectively (*p* = 0.40). The remaining 14 variants were classified as pathogenic or likely pathogenic, including 11 predicted loss-of-function variants and 3 missense variants (Figure 1). Importantly, whereas loss of function variants can often be interpreted sole on the basis of predicted effect on DNA sequence, missense variants require prior evidence in the literature for disease association. The 3 missense variants with adequate evidence based on published pedigrees and segregation to support classification as pathogenic or likely pathogenic were the p.Arg141Gln variant in *TNNI3* predisposing to hypertrophic cardiomyopathy and the p.Trp87Gly and p.Trp483Arg variants in *LDLR* predisposing to familial hypercholesterolemia, present in participants P6, P7, P8, and P10, respectively (32–36). Additional details on evidence used in

**FIGURE 1** Filtration and Classification of Variants Identified by Whole-Exome Sequencing in the Sudden Cardiac Death Case-Control Cohort

\*Missense variants filtered out if present with allele frequency  $\geq 0.0001$  in any racial subpopulation of the Genome Aggregation Database, a publicly available allele frequency database derived from 123,136 exome sequences (30). †Predicted loss-of-function variants do not necessarily all meet current standards to be classified as pathogenic or likely pathogenic. Representative examples include variants in the gene encoding titin (*TTN*) that occur in exons not found in cardiac-specific isoforms (44), variants in the gene encoding apolipoprotein B (*APOB*) that are associated with lower risk of hypercholesterolemia and coronary artery disease (45), and variants in the gene encoding tyrosine-protein phosphatase (*DSP2*), where only those variants that have previously been proven to segregate with disease are classified as pathogenic or likely pathogenic (3). ‡The ClinVar database provides open access to variant classifications shared by many individuals and clinical laboratories. Although it is an important means of communicating and coordinating efforts, many variant pathogenicity assertions in ClinVar do not warrant this designation based on current American College of Medical Genetics/Association of Molecular Pathology criteria (46).

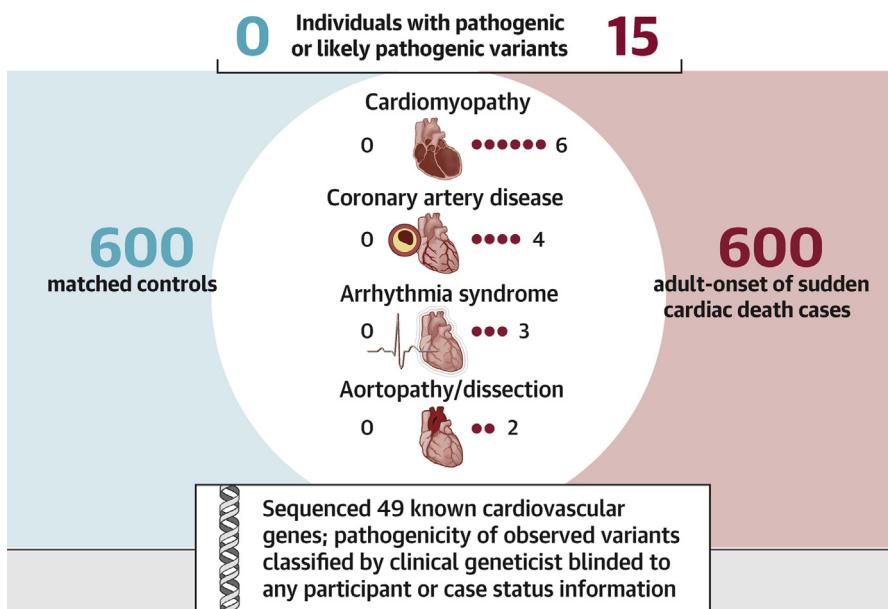
support of these annotations for each of the 14 pathogenic or likely pathogenic variants is provided in [Online Table 4](#).

A total of 15 individuals harbored 1 of these 14 variants, with 13 variants observed in only 1 individual and the p.Trp87Gly *LDLR* missense variant observed in 2 individuals, participants P7 and P8. Subsequent unblinding of sudden cardiac death status revealed that all 15 (100%) of the individuals harboring a pathogenic or likely pathogenic variant were sudden cardiac death cases and none were

controls. This represented a pathogenic or likely pathogenic variant prevalence of 2.5% (95% CI: 1.4 to 4.1%) in cases and 0% (95% CI: 0 to 0.6%) in controls ( $p < 0.0001$ ). Baseline characteristics among sudden cardiac death cases in whom a pathogenic or likely pathogenic variant was identified were similar to the remainder of the cases with respect to age, sex, and history of cardiovascular disease ([Online Table 5](#)). Of the 15 individuals harboring a pathogenic or likely pathogenic variant who experienced sudden cardiac death, 8 (53%) had no prior diagnosis of

**CENTRAL ILLUSTRATION** Gene Sequencing To Identify Pathogenic Variants in Sudden Cardiac Death Cases Versus Controls, and in Asymptomatic Adults

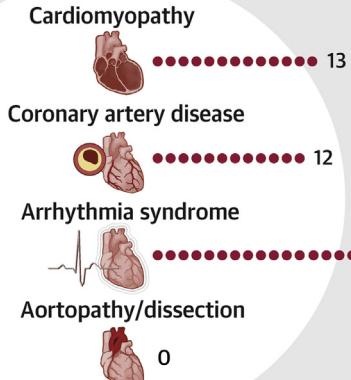
A. Prevalence of pathogenic or likely pathogenic variants in sudden cardiac death cases versus controls



4,525  
asymptomatic adults

0.9%  
41 of 4,525 with  
likely pathogenic or  
pathogenic variants

3.2X  
Increased risk  
of cardiovascular  
deaths over median  
follow-up of 14.3 yrs



B. Prevalence of pathogenic or likely pathogenic variants in asymptomatic adults and association with cardiovascular death in prospective follow-up

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(A) In an adult-onset sudden cardiac death case-control cohort, whole-exome sequencing identified pathogenic or likely pathogenic variants in 15 (2.5%) cases versus 0 (0%) controls ( $p < 0.0001$ ). The number of carriers of a pathogenic or likely pathogenic variant observed in each of 4 important conditions underlying sudden cardiac death is displayed according to case versus control status. Test of statistical significance was performed for the combined variant counts using the Fisher exact test. (B) Among 4,525 asymptomatic adult participants of the Multi-Ethnic Study of Atherosclerosis free of known cardiovascular disease, whole-genome sequencing identified 41 (0.9%) with a pathogenic or likely pathogenic variant—the number of carriers of a pathogenic or likely pathogenic variant observed in each of 4 important conditions underlying sudden cardiac death is displayed. Over a median follow-up of 14.3 years, these 41 carriers had a 3.24-fold ( $p = 0.02$ ) increased risk of incident cardiovascular death, as assessed in a Cox proportional hazards model adjusted for age, sex, and race.

**TABLE 2** Sudden Cardiac Death Case/Control Status and Clinical Characteristics of Carriers of a Pathogenic or Likely Pathogenic Mutation

Case ID	Age* and Sex	Case/Control	Known ASCVD†	Known CHF‡	Variant§	Gene (Variant Type)	Amino Acid or cDNA Change	Associated Disease	Classification Using ACMG Criteria
Cardiomyopathy variants									
P1	64 M	Case	No	No	2:179417587:C>A	TTN Premature stop	p.Gly27446Ter	Dilated cardiomyopathy	Likely pathogenic
P2	81 M	Case	No	Yes	2:179433438:G>T	TTN Premature stop	p.Tyr23239Ter	Dilated cardiomyopathy	Likely pathogenic
P3	64 F	Case	Yes	No	2:179444577:T>G	TTN Splice site	c.59645-2A>C	Dilated cardiomyopathy	Likely pathogenic
P4	75 M	Case	No	No	2:179486244:G>A	TTN Premature stop	p.Arg12535Ter	Dilated cardiomyopathy	Likely pathogenic
P5	61 M	Case	No	Yes	2:179604601:TTC>T	TTN Frameshift	p.Glu4215ArgfsTer7	Dilated cardiomyopathy	Likely pathogenic
P6	78 F	Case	No	No	19:55665525:C>T	TNNI3 Missense	p.Arg141Gln	Hypertrophic cardiomyopathy	Likely pathogenic
Coronary artery disease variants									
P7	78 M	Case	Yes	No	19:11213408:T>G	LDLR Missense	p.Trp87Gly	Familial hypercholesterolemia	Pathogenic
P8	70 M	Case	No	No	19:11213408:T>G	LDLR Missense	p.Trp87Gly	Familial hypercholesterolemia	Pathogenic
P9	69 F	Case	Yes	Yes	19:11221435:C>T	LDLR Premature stop	p.Arg350Ter	Familial hypercholesterolemia	Pathogenic
P10	73 F	Case	No	No	19:11224299:T>C	LDLR Missense	p.Trp483Arg	Familial hypercholesterolemia	Likely pathogenic
Arrhythmia syndrome variants									
P11	53 F	Case	No	No	6:7580603:C>T	DSP Premature stop	p.Gln1394Ter	Arrhythmogenic right ventricular cardiomyopathy	Likely pathogenic
P12	60 M	Case	No	No	7:150647424:G>A	KCNH2 Premature stop	p.Arg744Ter	Long QT syndrome	Pathogenic
P13	67 M	Case	Yes	Yes	7:150655169:CG>C	KCNH2 Frameshift	p.Pro298ArgfsTer62	Long QT syndrome	Likely pathogenic
Aortopathy/aortic dissection variants									
P14	75 F	Case	No	No	2:189855742:C>T	COL3A1 Premature stop	p.Arg271Ter	Vascular Ehlers-Danlos syndrome	Pathogenic
P15	80 F	Case	Yes	No	15:48892335:C>T	FBN1 Splice site	c.442+1G>A	Marfan syndrome	Likely pathogenic

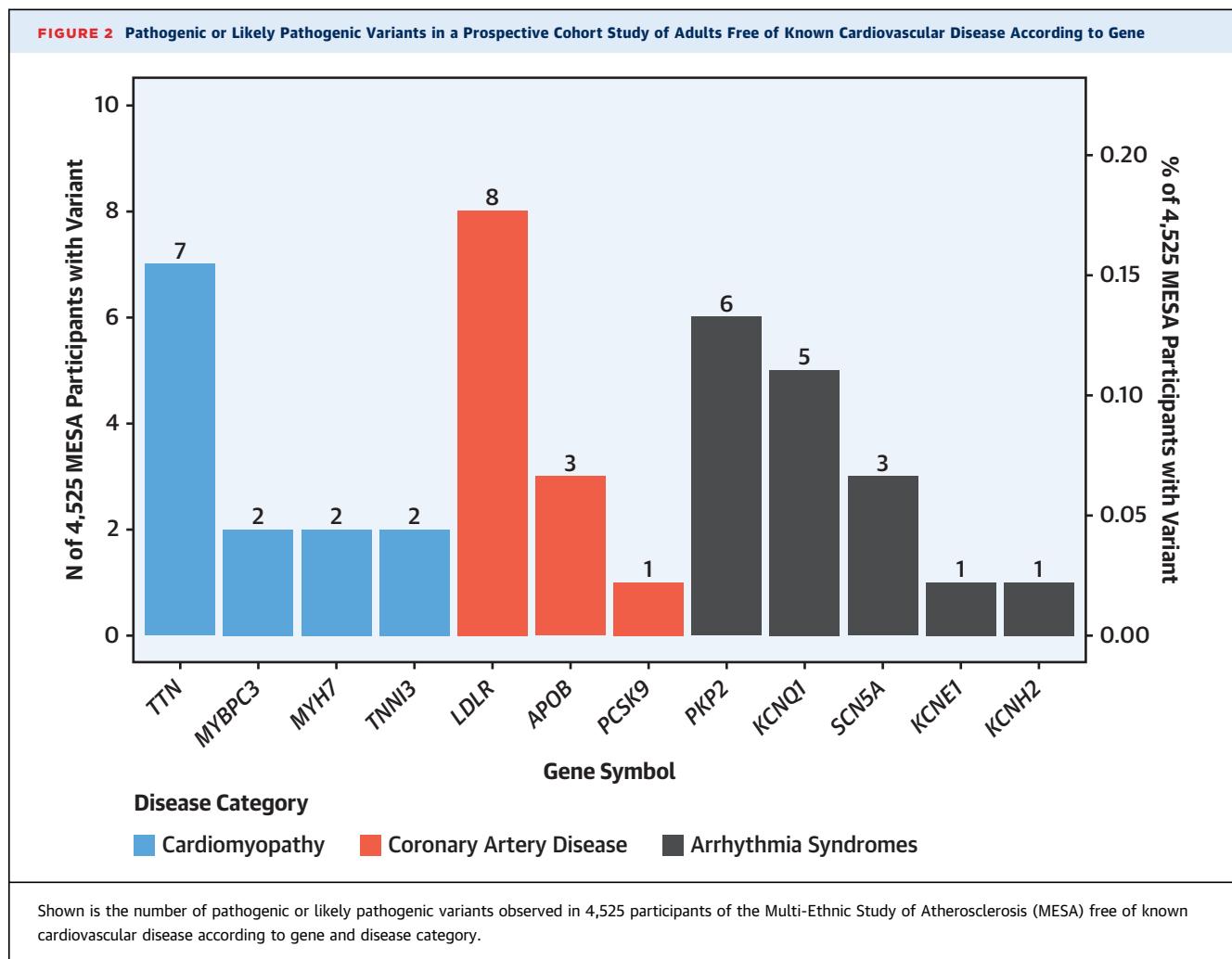
\*Age refers to age at sudden cardiac death event. †Known ASCVD before sudden cardiac death based upon review of medical records. ‡Known CHF or cardiomyopathy before sudden cardiac death based upon review of medical records. §Variant is described based on "chromosome:position reference allele>alternate allele" formatting, with chromosome positions based on the GRCh37 genome assembly.

ACMG = American College of Medical Genetics; ASCVD = atherosclerotic cardiovascular disease; CHF = congestive heart failure; ID = identity.

atherosclerotic cardiovascular disease or CHF. These 15 cases included 6 with a variant related to cardiomyopathy, 4 related to coronary artery disease, 3 related to an inherited arrhythmia syndrome, and 2 related to aortopathy or aortic dissection (**Central Illustration**). Variants in the *TTN* gene predisposing to dilated cardiomyopathy and the *LDLR* gene related to familial hypercholesterolemia were the most common, present in 5 and 4 sudden cardiac death cases, respectively (**Table 2**).

Although definitive confirmation that a given variant was the "cause" of a sudden cardiac death is typically not possible, we next reviewed available health records of the 15 cases that harbored a pathogenic or likely pathogenic variant to assess

concordance of the variant and mechanism of death. Of these, 6 of 15 (40%) were deemed probably consistent, 2 (13%) possibly consistent, and 7 (47%) uncertain (**Online Table 6**). Representative examples of probably consistent annotations include a history of CHF and ventricular tachycardia in a participant harboring a *TTN* cardiomyopathy variant (participant P2) and a history of lipid-lowering medication use and exertional angina in a participant harboring a *LDLR* familial hypercholesterolemia variant (participant P10). Other scenarios—such as a report of neck discomfort leading up to sudden cardiac death in a participant with a *FBN1* aortopathy/aortic dissection-variant were deemed possibly consistent (participant P15). Cases were deemed uncertain due to



lack of autopsy or relevant tests performed as part of clinical care before sudden cardiac death event.

Through analysis of the same 49 genes from whole-genome sequencing data and using an identical variant classification procedure, we next determined the prevalence of pathogenic or likely pathogenic variants in 4,525 adults free of known cardiovascular disease derived from the MESA prospective cohort study. Average participant age was 61 years (range 44 to 84 years) and 2,200 (49%) were male. A total of 1,837 (41%) of the participants were white, 1,098 (24%) black, 1,004 (22%) Hispanic, and 586 (13%) Asian.

A total of 15,348 variants were present in any of the 49 genes in at least 1 of the 4,525 MESA participants. This list was narrowed by bioinformatics filtering to 5,419 variants. A clinical laboratory geneticist who was blinded to phenotype status evaluated these 5,419 variants, and classified 39 as pathogenic or likely pathogenic according to ACMG/AMP criteria, including 15 predicted loss-of-function variants and

24 missense variants (Online Figure 2). Only 1 of these 39 variants, a splice site mutation in the TTN gene predisposing to cardiomyopathy, overlapped with the variants observed in the sudden cardiac death nested case-control dataset. A summary of the evidence used in support of these annotations for each of these 39 variants is provided in Online Table 6.

The 39 variants occurred in 12 distinct genes and, in aggregate, were present in 41 (0.91%) of the MESA participants (Figure 2). Individuals harboring these variants included 13 participants with a mutation related to cardiomyopathy, 12 related to coronary artery disease, 16 related to an inherited arrhythmia syndrome, and 0 related to aortopathy or aortic dissection (Central Illustration).

Cardiovascular death occurred in 165 of 4,525 (3.6%) participants over a median follow-up of 14.3 years (Q1-Q3: 13.7 to 14.8 years), including 4 of 41 (9.8%) with a pathogenic or likely pathogenic variant and 161 of 4,483 (3.6%) without such a variant

(Figure 3). In a survival analysis adjusted for age, sex, and race, participants with a pathogenic or likely pathogenic variant were at significantly increased risk of cardiovascular death compared to noncarriers (hazard ratio: 3.24; 95% CI: 1.20 to 8.79;  $p = 0.02$ ).

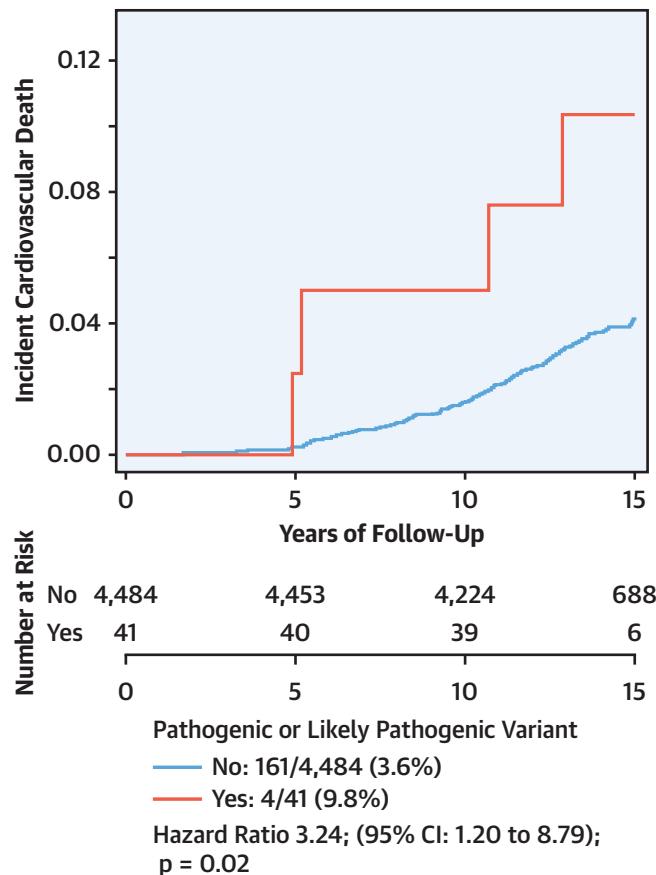
We next assessed the extent to which pathogenic variants associated with expected intermediate phenotypes. Among 12 carriers of a familial hypercholesterolemia mutation predisposing to coronary artery disease, average LDL cholesterol was 165 mg/dl versus 124 mg/dl in the remainder of the population ( $p = 0.01$ ), and 9 of the 12 (75%) met clinical criteria for hypercholesterolemia as compared with 45% of noncarriers ( $p = 0.04$ ). Among 8 carriers of a long QT syndrome variant predisposing to arrhythmia, mean QT interval corrected for heart rate was 457 ms versus 417 ms in the remainder of the population ( $p = 0.01$ ) and 4 of the 8 (50%) met clinical criteria for prolonged QT interval versus 4.2% in the remainder of the population ( $p = 0.0002$ ). Left ventricular ejection fraction was available in 4 patients with dilated cardiomyopathy mutations—mean value was 64% in mutation carriers versus 69% in the remainder of the population ( $p = 0.31$ ), with no carriers meeting clinical criteria for reduced ejection fraction. Among 3 carriers of a hypertrophic cardiomyopathy mutation, mean ejection fraction was 79% versus 69% in the remainder of the population ( $p = 0.04$ ), with all 3 carriers (100%) meeting clinical criteria for hyperdynamic contractile function versus 29.4% of the remainder of the population ( $p = 0.03$ ). Taken together, these results suggest that clinical assessment of asymptomatic carriers of pathogenic variants is likely to reveal high-risk intermediate features in a meaningful portion of individuals.

## DISCUSSION

In this study, we show that pathogenic or likely pathogenic variants in any of 49 cardiovascular disease genes are present in a small but potentially important subset of adults experiencing sudden cardiac death. Second, we determine that such variants are present in approximately 1% of an adult population free of known cardiovascular disease and conferred a more than 3-fold increased risk of cardiovascular death in prospective follow-up.

This analysis builds upon previous efforts to study the genetic basis of sudden cardiac death in several key ways (11–15). First, we emphasize the careful adjudication of sudden cardiac death in 600 adults from prospective cohort studies and the largest gene sequencing analysis of this phenotype to date. Second, cases were ascertained solely based upon

**FIGURE 3** Association of Pathogenic or Likely Pathogenic Variant Carrier Status With Incident Cardiovascular Death in a Prospective Cohort Study of Adults Free of Known Cardiovascular Disease



Among 4,525 adult participants of MESA (Multi-Ethnic Study of Atherosclerosis), whole-genome sequencing identified 41 (0.9%) with a pathogenic or likely pathogenic variant. Over a median follow-up of 14.3 years, these 41 carriers had a 3.24-fold (95% confidence interval [CI]: 1.20 to 8.79;  $p = 0.02$ ) increased risk of incident cardiovascular death, as assessed in a Cox proportional hazards model adjusted for age, sex, and race.

experiencing sudden cardiac death, eliminating the potential referral bias caused by the requirement of an unrevealing autopsy or referral from a medical examiner. Third, a certified clinical laboratory geneticist performed clinical-grade assessment of variant pathogenicity in both cases and matched controls blinded to any phenotype information. Fourth, we extend our variant annotation approach used in our sudden cardiac death nested case control analysis to a population-based sample of 4,525 asymptomatic adults.

Gene sequencing to identify individuals with a pathogenic variant at scale in the population is increasingly practical as the costs of sequencing have declined rapidly. At least 1 U.S. health care system has

committed to returning pathogenic variants related to cardiovascular disease to patients and their health care providers (4), and a similar commitment has been embraced as a core tenet of the All of Us Research Program, a U.S. National Institutes of Health sponsored study that plans to enroll >1 million participants in coming years (37).

Explicit demonstration that gene sequencing reduces the risk of sudden cardiac death is unlikely to be attainable given low absolute event rates. However, identification of pathogenic mutation carriers before disease onset may already provide a clinically actionable opportunity for targeted screening or prevention. Each of the 4 sudden cardiac death etiologies considered have a strong evidence base for treatment considerations, ranging from aggressive LDL cholesterol lowering for those with familial hypercholesterolemia to beta-blocker therapy for those with long QT syndrome (6-10). Below, we highlight several key challenges to widespread implementation into clinical practice.

First, the current variant classification procedure may underestimate pathogenic variant prevalence, deeming some variants as of uncertain significance because they currently have insufficient evidence for disease association. This is because assessing the pathogenicity of a given missense variant relies heavily on previous observations of the variant in family-based studies and co-segregation with disease and detailed manual curation of this evidence (17,18). The labor-intensive nature of clinical-grade variant classification remains an important barrier to rapid scaling of genome interpretation for large segments of the population. However, efforts are ongoing to enhance variant annotation by incorporating larger and more diverse population allele frequency databases (38), promote data sharing and best practices (18,39), and develop new technology or machine learning algorithms to enable high-throughput functional assessment of variants in a given gene (40,41).

Second, current clinical care is largely designed to treat patients with disease, not healthy individuals with inherited susceptibility. Few health systems house the necessary physician and genetic counseling expertise to effectively manage healthy individuals who harbor pathogenic risk variants. A larger workforce trained in both genome interpretation as well as the psychosocial or health care cost implications of genetic risk disclosure is needed to realize the full potential of genomic medicine (1).

Third, current guidelines to report variants as "pathogenic" may lead to misinterpretation by patients and their health care providers as a pre-determined destiny to manifest disease, but

whether genetic risk ultimately manifests itself as disease is related to a large number of genetic and nongenetic factors (17). For example, although familial hypercholesterolemia variants confer a 4-fold increased risk of coronary artery disease, approximately 25% of individuals who harbor such a variant do not meet clinical criteria for hypercholesterolemia (42). Similarly, despite conferring a more than 10-fold increased risk of dilated cardiomyopathy, many individuals with a loss-of-function *TTN* mutation have a normal left ventricular ejection fraction (43). We thus suggest improving genetic risk disclosure by describing the risk conferred by variants in a probabilistic manner rather than a dichotomy of pathogenic versus not.

**STUDY LIMITATIONS.** First, our analysis, as with previous gene sequencing studies of sudden cardiac death, was limited in the ability to definitively link an individual's demise to an identified pathogenic or likely pathogenic variant (11-15). However, the enrichment of such variants in sudden cardiac death cases as compared to a matched control group suggests significant attributable risk. Second, our sudden cardiac death case-control cohort was comprised primarily of white individuals. Third, individual pathogenic or likely pathogenic variants are exceedingly rare and were thus considered in aggregate to confirm enrichment in sudden cardiac death cases. Fourth, our analysis of asymptomatic adults free of known cardiovascular disease was performed in the middle-aged MESA cohort, raising the potential for an underestimation of pathogenic variant prevalence due to survival bias. Fifth, the association of pathogenic variants with intermediate risk features in MESA participants was limited to phenotypes available in the cohort.

## CONCLUSIONS

Gene sequencing confirms significant enrichment of pathogenic variants in sudden cardiac death cases as compared to controls, and such variants are present in approximately 1% of asymptomatic adults free of known cardiovascular disease. We believe these results lay the scientific foundation for the integration of gene sequencing to identify asymptomatic individuals with pathogenic variants into routine clinical practice, with the ultimate goal of preventing a sudden cardiac death tragedy that might have been anticipated.

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## PERSPECTIVES

### COMPETENCY IN MEDICAL KNOWLEDGE:

Gene sequencing identifies pathogenic variants in a small subset of adults experiencing sudden cardiac death that are also present in ~1% of asymptomatic individuals.

**TRANSLATIONAL OUTLOOK:** Additional research is needed to assess the utility of integrating genetic testing into risk assessment algorithms for prevention of sudden cardiac death in adults.

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**KEY WORDS** gene sequencing, genomic medicine, sudden cardiac death

**APPENDIX** For supplemental Methods, figures, and tables, please see the online version of this paper.