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ORIGINAL RESEARCH

PACING THERAPY

Rare Genetic Variants in Young Adults Requiring Pacemaker Implantation

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ABSTRACT

BACKGROUND Genetic disease has recently emerged as a cause of cardiac conduction disorders (CCDs), but the diagnostic yield of genetic testing and the contribution of the different genes to CCD is still unsettled.

OBJECTIVES This study sought to determine the diagnostic yield of genetic testing in young adults with CCD of unknown etiology requiring pacemaker implantation. We also studied the prevalence of rare protein-altering variants across individual genes and functional gene groups.

METHODS We performed whole exome sequencing in 150 patients with CCD of unknown etiology who had permanent pacemaker implanted at age ≤60 years at 14 Spanish hospitals. Prevalence of rare protein-altering variants in patients with CCD was compared with a reference population of 115,522 individuals from gnomAD database (control subjects).

RESULTS Among 39 prioritized genes, patients with CCD had more rare protein-altering variants than control subjects (OR: 2.39; 95% CI: 1.75-3.33). Significant enrichment of rare variants in patients with CCD was observed in all functional gene groups except in the desmosomal genes group. Rare variants in the nuclear envelope genes group exhibited the strongest association with CCD (OR: 6.77; 95% CI: 3.71-13.87). Of note, rare variants in sarcomeric genes were also enriched (OR: 1.73; 95% CI: 1.05-3.10). An actionable genetic variant was detected in 21 patients (14%), with *LMNA* being the most frequently involved gene (4.6%).

CONCLUSIONS Unrecognized rare genetic variants increase the risk of CCD in young adults with CCD of unknown etiology. Genetic testing should be performed in patients age ≤60 years with CCD of unknown etiology. The role of genetic variants in sarcomeric genes as a cause of CCD should be further investigated.
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Cardiac conduction disorders (CCDs) can appear as a consequence of several heart diseases such as ischemic cardiac disease, congenital heart disease, cardiomyopathies, myocarditis, infiltrative diseases, and idiopathic degeneration of cardiac conduction tissue.[1] Although the underlying condition has a strong impact on both treatment and prognosis, the specific etiologies of CCD are commonly not identified in adults during routine clinical practice. Although conduction system degeneration and fibrosis are thought to be the leading cause of CCD in the elderly,[2] the underlying etiology of CCD in the young and middle-aged population remains virtually unknown.[3]

Recently, it has been reported that cardiac conduction abnormalities exhibit familial association[4] and that may occur in relation with rare genetic variants.[4,5] Several genes have been described to be related with CCD, with conduction abnormalities being the unique manifestation of the disease, or in the context of a more complex presentation with CCD associated with cardiomyopathy,[6] congenital abnormalities,[7] or extracardiac disorders such as neuromuscular diseases.[8]

Although the prevalence and etiology of CCD in young patients requiring permanent pacemaker (PPM) implantation has been described in a few cohorts, the contribution of genetics has not been systematically evaluated.[9] Consequently, the diagnostic yield of genetic testing and the contribution of the different genes to the phenotype in young adults with advanced CCD requiring PPM implantation is still unclear.

This study sought to determine the contribution of genetics and the usefulness of genetic testing in young adults with advanced CCD of unknown etiology requiring PPM implantation.

METHODS

STUDY POPULATION. This was a multicenter study of individuals with advanced CCD who underwent PPM implantation at 14 Spanish hospitals between 2015 and 2021. PPM databases at participating institutions were reviewed and adult patients age ≤60 years at the time of PPM implantation were invited to participate if no cause for CCD had been identified. Therefore, patients with a secondary cause that could explain the disease, such as radiotherapy, infections (Chagas, borrelia, and so on), or PPM implantation in the context of acute myocardial infarction or cardiac surgery were excluded. Diagnosis of a cardiomyopathy at the time of PPM implantation was also considered an exclusion criterion; this included the presence of a left ventricle maximal wall thickness (LVMWT) ≥15 mm for hypertrophic cardiomyopathy (HCM) and a left ventricular ejection fraction (LVEF) ≤45% for dilated cardiomyopathy (DCM).

Familial data were obtained after a structured interview and a familial pedigree was drawn. Demographics, symptoms, 12-lead electrocardiogram, and transthoracic echocardiogram data at first evaluation at participating centers were gathered from clinical records using uniform methodology. A family history of PPM implantation was considered if one or more relatives of any age (in addition to the proband) had a PPM implanted.

The study was approved by Hospital Universitario Puerta de Hierro ethics committee and conformed to the principles of the Helsinki Declaration. The authors from each participating center guarantee the integrity of data.

ABBREVIATIONS AND ACRONYMS

ACMG = American Collage of Medical Genetics

ARVC = arrhythmogenic right ventricular cardiomyopathy

AVB = atrioventricular block

CCD = cardiac conduction disorder

DCM = dilated cardiomyopathy

HCM = hypertrophic cardiomyopathy

LVEF = left ventricular ejection fraction

LVMWT = left ventricle maximal wall thickness

MAF = minor allele frequency

PCCD = progressive cardiac conduction disease

PPM = permanent pacemaker

SND = sinus node dysfunction

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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CCD GENES. We selected a group of 39 genes definitely or possible related with CCD according to current recommendations and the current evidence from a comprehensive published data review.[10,11] Genes were clustered into 6 functional gene groups based on similar common functions, involvement in biological processes, localization to subcellular compartments, and other shared properties based on consolidated scientific evidence from the literature and available biological databases. Functional gene groups included the following:

1. Channels: *CACNA1C, CACNA1D, GJA5, HCN4, KCNH2, KCNJ2, KCNK17, KCNQ1, RYR2, SCN10A, SCN1B, SCN4B, SCN5A, TRPM4*.
2. Desmosomal: *DSC2, DSG2, DSP, PKP2*.
3. Nuclear envelope: *EMD, LMNA*.
4. Sarcomeric: *ACTC1, MYBPC3, MYH6, MYH7, TNNC1, TNNI3, TNNT2*.
5. Regulatory: *GATA6, NKX2-5, NPPA, TBX20, TBX5, TNNI3K*.
6. Other genes: *ANK2, DES, GLA, LAMP2, PRKAG2, TTR*.

WHOLE EXOME SEQUENCING AND VARIANT ANALYSIS. The study was performed using a multiple approach based on targeted Next Generation Sequencing (NGS) combined with the gold standard Sanger technique. Blood samples were subjected to automated genomic DNA purification (QIAsymphony SP, Qiagen). Library preparation was carried out using the SureSelectXT Reagent library preparation kit (Agilent) for Illumina paired-end multiplexed sequencing method. Enrichment was performed using a custom Clinical Research Exome (Agilent), which selectively captures coding regions and adjacent intronic areas (± 10 bp) for the selected genes. After cluster generation on a cBot (Illumina), captured DNA was sequenced on the Illumina HiSeq-1500 platform. Low-coverage regions in selected genes were tested in parallel by standard Sanger sequencing. Bioinformatics analysis was performed by means of a custom pipeline that includes software such as NovoAlign, GATK (Genome Analysis Tool Kit), SAMTools, and Bcftools for variant calling and genotyping. First, the NGS reads were subjected to quality control checks for removing any low-quality reads. Then the reads were mapped (aligned) to GRCh37. ANNOVAR software was used to functionally annotate detected variants. The list of validated variants was further trimmed by omitting those reported in dbSNP, or present in gnomAD database with a minor allele frequency (MAF) ≥1%. Variant deleteriousness was predicted using different web-based tools: Polyphen-2, SIFT, MutationTaster, and DANN. For predicting splice-site variants effect, 4 different software tools were used: NNSplice, MaxEntScan, HSFHuman Splicing Finder, and SpliceAI.

ENRICHMENT OF THE VARIANTS IN THE CCD COHORT. Allele frequencies in the general population were extracted from the gnomAD database version 2.1.1, August 2022. We applied an MAF threshold of 5×10^{-5} to consider a variant in a gene as a potential candidate (very rare variant). Moreover, we also excluded variants with a MAF $\geq 1 \times 10^{-4}$ in any subpopulation of gnomAD to avoid that variants could be enriched in 1 specific population.[12] The enrichment in CCD genes was determined comparing the prevalence of variants in the CCD cohort with the prevalence in the control population of gnomAD, obtaining the OR and the *P* value as previously described.[13]

Additionally, we determined the prevalence of clinically actionable genetic variants in CCD genes. To do so, we evaluated the pathogenicity of the potentially relevant variants identified according to the American College of Medical Genetics and Genomics (ACMG) as detailed in Supplemental Methods.[14] A variant was considered clinically actionable if it was considered pathogenic (P) or likely pathogenic (LP) (therefore, both considered disease-causing), or if it was classified as a "hot variant of unknown effect (VUS)."[15] A variant was classified as a "hot VUS" if the following: 1) exhibited a high score according to ACMG classification, but the LP criteria was not reached (1 strong + 1 supporting OR 2 moderate and 1 supporting OR 1 moderate and ≥2 supporting, without any criteria of benignity); AND 2) further evaluation in the proband and the family (cosegregation) were recommended after discussion by a multidisciplinary team.

We also evaluated the yield of genetic testing, according to type of CCD as classified in European Heart Rhythm Association/Heart Rhythm Society/Asia Pacific Heart Rhythm Society/Latin American Heart Rhythm Society consensus statement on the state of genetic testing for cardiac diseases.[16] Accordingly, patients were divided in 2 groups: progressive cardiac conduction disease (PCCD) and sinus node dysfunction (SND). PCCD group included the following: trifascicular block (first-degree atrioventricular block [AVB] + left bundle branch block; or first degree AVB + right bundle branch block + left anterior hemiblock), second- and third-degree AVB. SND group included the following: sinus node disease, extreme bradycardia, and sinus pauses >6 seconds.

STATISTICAL ANALYSIS. Continuous variables were expressed as mean ± SD, and comparison between groups was performed using the Student's *t*-test or the Mann-Whitney *U* test according to whether or not the values were normally distributed. Categorical variables were expressed in a contingency table as number of observations for each category or group (and their relative frequencies over each group) and compared using the chi-square test or Fisher exact test if one or more of the expected frequencies in any cell was below 5. ORs were estimated from a 2 × 2 contingency table as OR = (cases with variant × control subjects without variant)/(cases without variant × control subjects with variant) and the corresponding 95% CIs through the exact method, as previously described.[13] A 2-sided *P* value <0.05 was considered to indicate statistical significance. Analysis was performed using the R version 4.0.4 (The R Foundation for Statistical Computing Platform).

RESULTS

A total of 153 patients were initially evaluated to participate in the study. Three patients were excluded because they met criteria for HCM with an LVMWT ≥15 mm upon review of submitted data at the coordinating center. Therefore, 150 patients who met inclusion criteria were included in the final analysis (**Figure 1**).

Baseline characteristics of these patients are shown in **Table 1**. Mean age at PPM implant was 53.3 ± 6.8 years and 38% were women. Of note, 21.3% of the patients were age <50 years when PPM was implanted. Prevalence of hypertension, dyslipidemia, and diabetes was 36.7%, 39.3%, and 17.3%, respectively. Family history of PPM implantation and of sudden cardiac death (SCD) were present in 17.4% and 10.7%, respectively. Syncope was the most common symptom before PPM implant (51.3%), followed by dizziness, dyspnea, and palpitations (36.7%, 15.3% and 10.7%, respectively). There was not any patient with a diagnosis/suspicion of myopathy, and only 2 patients had a diagnosis of neuropathy (both were diabetic, and no relevant variants were identified in their genetic study).

CCD leading to PPM implantation was SND in 43 subjects (28.6%) and PCCD in 107 (71.3%). Among the latter group, 12 individuals (8.0%) had trifascicular block, 24 (16%) second-degree AVB, and 71 (47.3%) complete AVB. There were no differences between patients with SND and PCCD in the age at PPM implantation (52.8 ± 6.6 years vs 53.5 ± 6.9 years; *P* = NS) and in the proportion of patients with PPM implanted with ≤50 years (9 of 43 [20.9%] vs 22 of 107 [20.6%]; *P* = NS).

ENRICHMENT OF VERY-RARE GENETIC VARIANTS IN CCD GENES. A total of 71 very-rare variants in CCD genes were identified in 60 (40%) individuals (9 patients harbored 2 very-rare variants). The complete list of variants with their final ACMG classification is shown in Supplemental Table 1. These variants were significantly enriched in patients with CCD and PPM implantation (71 of 150; 47.3%) compared to controls (31,276/115,522; 27.1%), with an OR=2.39 (95%CI:1.75-3.33; *P* < 0.0001). Variants were significantly enriched in all functional gene groups, except in the desmosomal genes group (**Figure 2** and Supplemental Table 2). Burden of rare variants in the nuclear envelope gene group exhibited the highest association with CCD compared with control subjects (OR: 6.77; 95% CI: 3.71-13.87), followed by regulatory genes (OR: 2.85; 95% CI:1.56-5.84), channels (OR: 1.84 (95% CI:1.25-2.85) and sarcomeric genes (OR: 1.73; 95% CI: 1.05-3.10).

The complete list of the 21 genes with at least 1 very-rare genetic variant identified in the CCD probands and their individual ORs compared with control subjects are shown in **Figure 2** and Supplemental Table 3. The strongest association was observed for *LMNA* (OR: 7.14; 95% CI: 3.68-16.08) and *ACTC1* (OR: 9.21; 95% CI: 2.75-70.60), followed by *EMD* (OR: 5.17; 95% CI: 1.85-22.81), *KCNJ2* (OR: 5.36; 95% CI: 1.92-23.61), and *GATA6* (OR: 3.80; 95% CI: 1.54-12.92). Of note, rare variants in *MYH7* (OR: 2.72; 95% CI: 1.34-6.49) and *TNNI3K* (OR: 2.52; 95% CI: 1.18-6.52) were also significantly enriched in CCD probands compared with control subjects.

CLINICALLY ACTIONABLE VARIANTS. Of the 150 patients included in the study, 21 (14%) had a clinically actionable variant. A P variant was identified in 8 patients (5.3%) and an LP variant in 9 (6.0%). Of the 17 individuals with a P/LP variant, 7 had a variant in *LMNA*, 2 in *MYH7*, and 8 patients had 1 variant in *ACTC1, EMD, SCN5A, MYBPC3, TRPM4, TNNT2, TTN,* and *TTR* each. Four additional patients (2.66%) had a variant classified as a "hot-VUS" in the following genes: *MYBPC3, MYH7, SCN5A*, and *TNNI3K* (**Figure 1** and Supplemental Table 1). Additionally, 48 VUS were identified in 42 probands (6 individuals had 2 VUS).

The proportions of patients with P/LP and with actionable variants (P/LP/hot-VUS) were similar between patients with SND and those with PCCD (14% vs 10.3% and 14% vs 14%, respectively).

LMNA was the gene with the higher number of clinically actionable variants, with 7 (2 were radical

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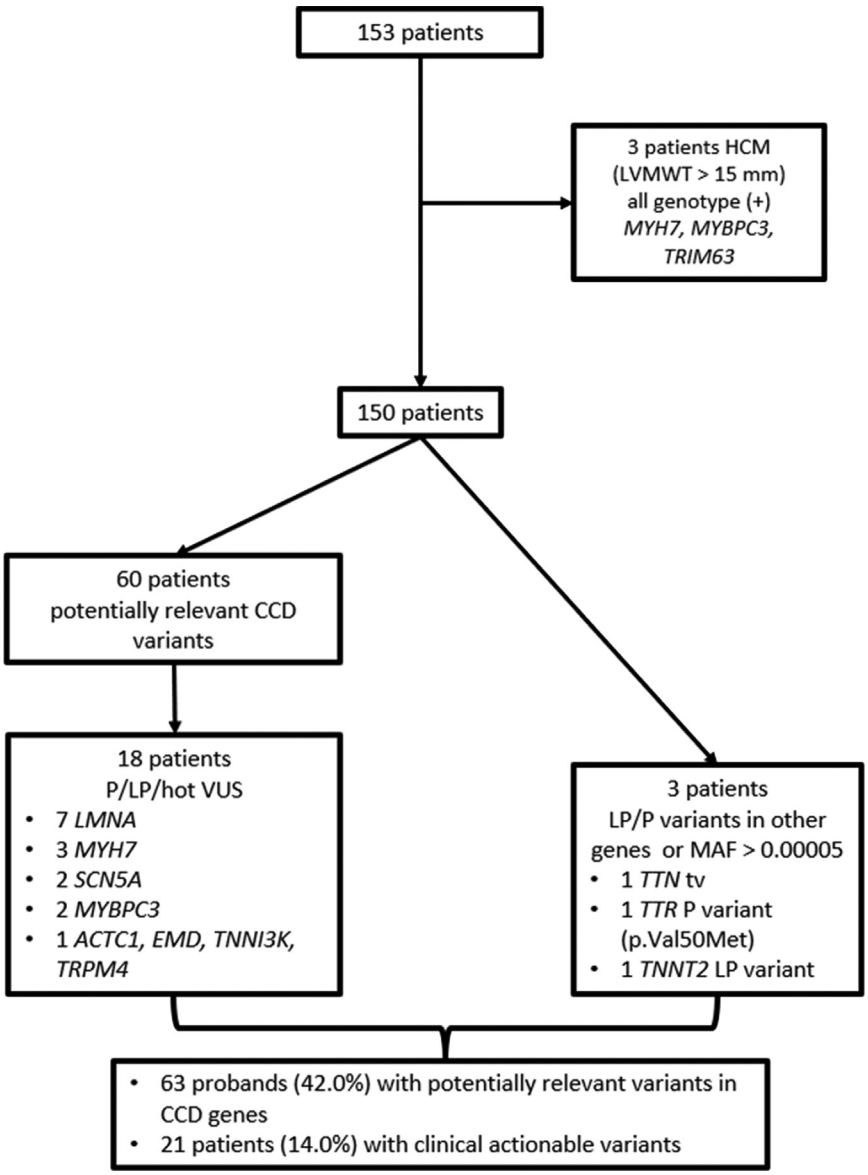
FIGURE 1 Flow Chart of the Study



CCD = cardiac conduction disorder; HCM = hypertrophic cardiomyopathy; LP = likely pathogenic; LVMWT = left ventricle maximal wall thickness; P = pathogenic; VUS = variant of unknown effect.

variants, truncating or affecting the transcription initiation codon, and 5 were missense alterations). A disease causing-variant in *EMD*, another gene of the nuclear envelope, was also identified in 1 subject. All of these individuals had normal LVEF and normal LVEDD, with the exception of 1 proband (carrier of the *LMNA* p.Arg439Cys variant) who had an LVEF in the low range of normality at initial evaluation (LVEF of 50%).

Six clinically actionable variants were identified in sarcomeric genes, with *MYH7* being the most frequent involved gene (3 probands), followed by *MYBPC3* (2 probands) and *ACTA1* (1 proband). None of these patients had a diagnosis of HCM, but 4 had mild LV hypertrophy (12-13 mm). Of the remaining variants, 3 variants were identified in genes encoding cardiac channels (2 *SCN5A* and 1 in *TRPM4*), and 1 in *TNNI3K* with evidence of cosegregation in the family.

TABLE 1 Baseline Characteristics of the Patients Included in the Study

	Overall (n = 150)	Noncarriers of CCD Rare Variants (n = 87)	Carriers of CCD Rare/Actionable Variants (n = 63)	Significance (*P* Value)
Female	57 (38)	33 (37.9)	24 (38.1)	NS
Age at PPM implant, y	53.3 ± 6.82	53.89 ± 6.43	52.46 ± 7.30	NS
Age at PPM implant ≤50 y	32 (21.3)	11 (12.6)	21 (33.3)	0.002
Hypertension	55 (36.7)	29 (33.3)	26 (41.3)	NS
Diabetes	26 (17.3)	14 (16.1)	12 (19.0)	NS
Dyslipidemia	59 (39.3)	31 (35.6)	28 (44.4)	NS
Atrial fibrillation	10 (6.7)	5 (5.7)	5 (8.1)	NS
Family history SCD	16 (10.7)	8 (9.3)	8 (12.7)	NS
Family history PPM	26 (17.4)	13 (15.1)	13 (20.6)	NS
Symptoms (pre-PPM implantation)				
Dizziness	55 (36.7)	28 (32.2)	2 (42.9)	NS
Palpitations	16 (10.7)	10 (11.5)	6 (9.5)	NS
Syncope	77 (51.3)	48 (55.2)	29 (46.0)	NS
Dyspnea (NYHA functional class ≥ II)	23 (15.3)	17 (19.5)	6 (9.5)	NS
PPM indication				
Sinus node dysfunction	43 (28.6)	25 (28.7)	18 (28.6)	NS
Progressive cardiac conduction disease	107 (71.3)	56 (64.3)	51 (80.9)	NS
Trifascicular block	12 (8.0)	2 (2.3)	10 (15.9)	NS
Second-degree AVB	24 (16.0)	14 (16.1)	10 (15.9)	NS
Third-degree AVB	71 (47.3)	40 (46.0)	31 (49.2)	NS
Echocardiography				
IVS, mm	9.62 ± 3.17	9.50 ± 3.13	9.80 ± 3.24	NS
PW, mm	8.91 ± 2.62	8.76 ± 2.52	9.12 ± 2.76	NS
EF, %	62.34 ± 7.79	61.63 ± .48	63.30 ± 8.15	NS
EF ≤50%	6 (4.4)	3 (3.8)	3 (5.2)	NS
EDLVD, mm	45.58 ± 10.9	45.79 ± 10.89	45.28 ± 11.16	NS

Values are n (%) or mean ± SD.

AVB = atrioventricular block; EF = ejection fraction; HCM = hypertrophic cardiomyopathy; IVS = intraventricular septum; LVEDD = left ventricular end-diastolic diameter; MLVWT = maximum left ventricular wall thickness; NS = not significant; PPM = permanent pacemaker; PW = posterior wall; SCD = sudden cardiac death.

Three additional clinically actionable variants in cardiovascular genes not detected in the enrichment analysis of very-rare genetic variants were identified. Two of them were variants present in control subjects with an MAF ≥5 × 10^{-5}: the pathogenic p.Val50Met in *TTR* associated with hereditary transthyretin amyloidosis, and the p.Arg278Cys variant in *TNNT2* that has been demonstrated to cause HCM.[17] The other variant was a truncating variant in *TTN* (p.Arg4334*) located in the I band of the protein but affecting the main cardiac isoform of the gene (N2B) considered to be pathogenic in association with DCM.

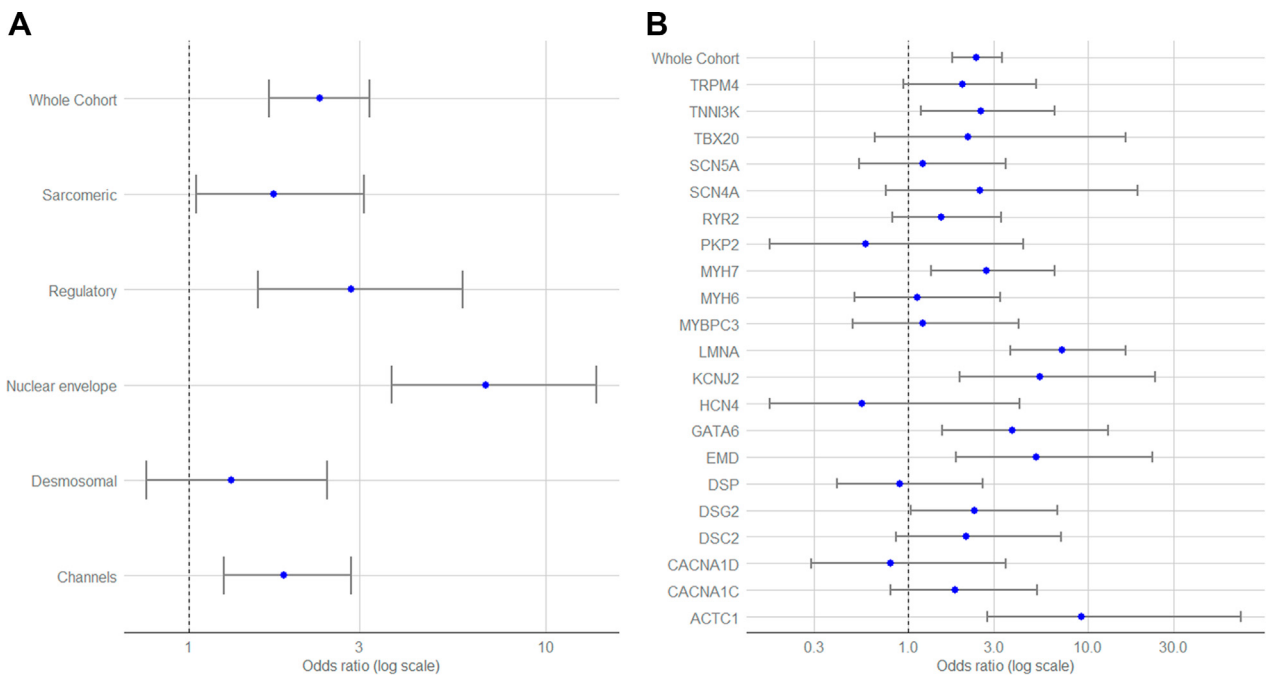
Carriers of clinically actionable variants exhibited a higher prevalence of a family history of PPM implantation and SCD compared with noncarriers (42.9% vs 13.3%; $P = 0.001$; and 23.8% vs 8.6%; $P = 0.037$, respectively).

We did not find an association between the presence of variants in functional gene groups among SND and PCCD groups (Supplemental Table 4).

AGE OF PPM IMPLANTATION ACCORDING TO GENETIC STATUS. A total of 63 probands (42% of the cohort) harbored at least 1 very rare and/or actionable genetic variant in a cardiovascular gene potentially associated with CCD, including 3 patients that had been excluded in the enrichment phase (Figure 1); 21 probands (14%) had an actionable variant (P/LP/hot VUS). There were no differences between probands harboring potentially relevant variants in CCD genes compared with noncarriers of these variants in terms of baseline characteristics, family history, symptoms, and PPM indication, except for the proportion of patients with implantation of PPM at age ≤50 years (33.3% vs 12.6%; $P = 0.002$) (Table 1).

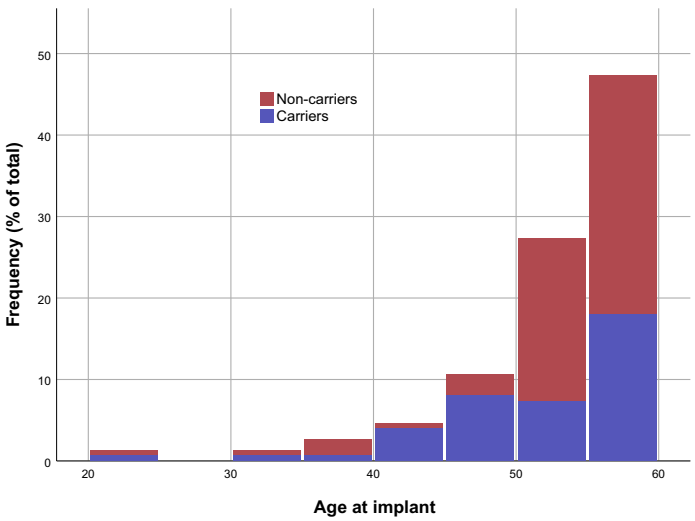
In fact, age at PPM implantation was an important determinant for the presence of clinically actionable genetic variants and the incidence of rare protein-altering variants decreased with age (Figure 3). The prevalence of rare protein-altering variants in patients with PPM implanted ≤50 years was 66.7% (21 of 32 probands) compared with 35.6% (42 of 118) in

FIGURE 2 Enrichment of Rare Genetic Variants by Functional Gene Groups and by Genes in Probands With Cardiac Conduction Disorder Compared With Control Subjects



(A) Forest plot of the enrichment of very-rare variant in genes by functional gene group compared with control subjects. (B) Forest plot of the enrichment of very rare variants in individual genes compared with control subjects (only autosomal dominant genes in which very rare variants were detected are shown). See Supplemental Tables 2 and 3 for more details.

FIGURE 3 Distribution of Probands With and Without Rare Genetic Variants According to Age



Distribution of probands according to age at permanent pacemaker implant. Carriers of very rare variants in cardiac conduction disorder genes are shown in blue, and noncarriers in red.

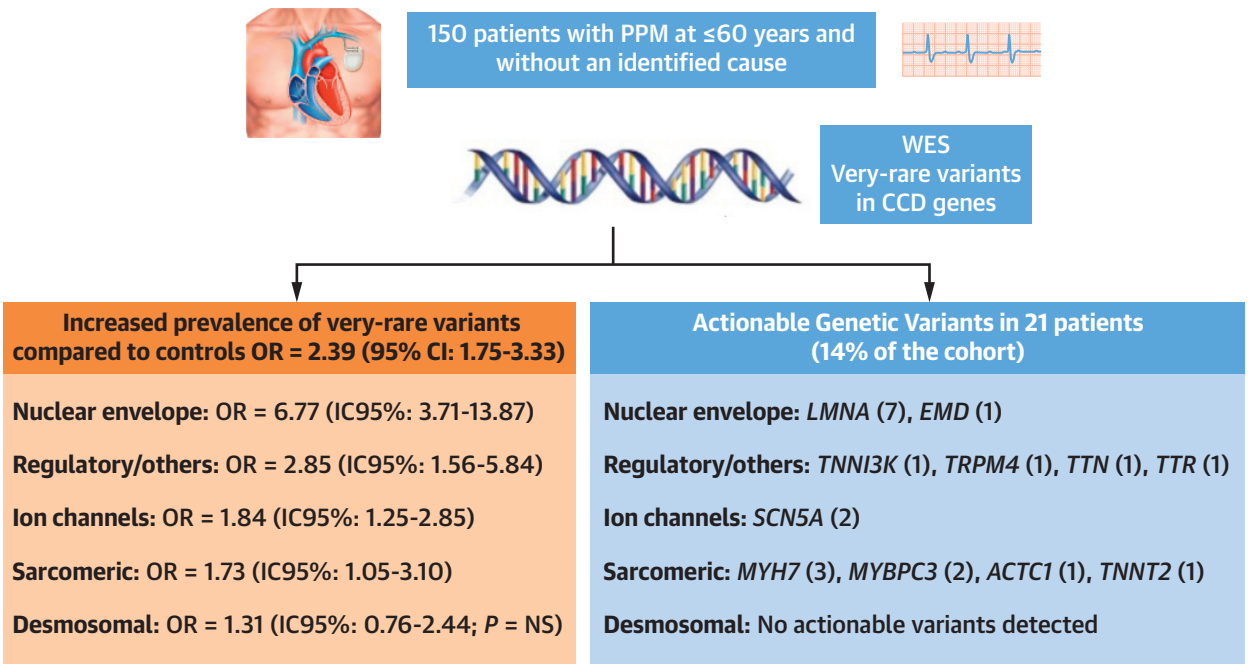
probands age >50 years ($P = 0.002$). The proportion of carriers of actionable variants was also higher in patients with PPM implanted at age ≤50 years (21.9%) than in patients with PPM implanted at age >50 years (11.9%) but this difference was not statistically significant ($P = 0.14$).

DISCUSSION

We demonstrate an increased prevalence of CCD-associated gene variants in young adults (age ≤60 years) with unexplained CCD requiring a PPM compared with control subjects (**Central Illustration**). The increased burden of rare protein-altering variants indicates that genetics is an important component in CCD susceptibility. Furthermore, a high proportion of probands (14%) harbored variants that can be considered "actionable variants," including disease-causing P/LP variants that can be used in cascade screening (11.3%), and hot-VUS that require further clinical evaluation of the patients or cosegregation studies in the family.

GENETIC TESTING AND CCD. Preliminary recent studies with a limited number of patients have highlighted the possible useful role of genetics to explain

CENTRAL ILLUSTRATION **Genetic Variants in Young Adults With Cardiac Conduction Disease Requiring Pacemaker Implantation**



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Genetic testing by whole exome sequencing (WES) of 150 patients with cardiac conduction disorder (CCD) of unexplained origin and permanent pacemaker (PPM) implanted at age ≤60 years showed increased prevalence of very-rare genetic variants in 39 cardiovascular genes both globally and in all functional gene groups except desmosomal gene groups compared with 115,522 individuals from the gnomAD database (control subjects). Additionally, 21 patients (14%) harbored actionable variants in disease-causing genes. Actionable variants were detected in genes from all functional gene groups except in desmosomal genes.

CCD in patients who require PPM implantation at a young age.[4,5] In an initial Italian single-center study of 15 individuals age ≤55 years at time of PPM implantation and who had ≥2 red flags for genetic heart disease, 4 patients had pathogenic variants in genes associated with channelopathies or cardiomyopathies and 6 exhibited VUS, while a recent study in South Switzerland of 15 patients age <50 years presenting with advanced AVB of unknown cause identified 1 pathogenic variant in 1 patient and 9 additional subjects harbored VUS.[5] A more numerous study from Denmark studied patients age <50 years with AVB of unknown etiology and found P/LP variants in 12 patients (5%).[18]

Our study expands these initial observations and confirms the critical role that genetics play in the etiology of CCD in young individuals, highlighting that a significant proportion of these patients have definitive familial disease with implications in medical care of the affected patients and their relatives. Unlike previously published studies, our study included patients with different forms of CCD, including SND and not only AVB, and expanded the age of PPM implantation to up to 60 years. Of note, 28.6% of patients included in our study had a PPM implanted for SND, and the proportion of patients with actionable variants among those with SND was similar to that in patients with PCCD.

Moreover, although patients age ≤50 years had higher prevalence of very rare and clinical actionable genetic variants (with prevalence of the latter above 20%), the prevalence among patients age >50 years was still remarkable (clinical actionable genetic variants in 11.9%). Accordingly, we believe that based in our results, genetic testing should be offered to all patients ≤60 years with CCD of unidentified origin requiring a PPM irrespective of the underlying CCD.

GENETIC ARCHITECTURE OF CCD. Our study is the first to examine the genetic architecture of CCD and the individual contribution of genes and gene groups. When we analyzed the groups of genes with a greater probability of association with CCD, genes that code for proteins located in the inner nuclear membrane

exhibited the highest association with CCD. Variants in *LMNA* had previously shown to be associated with CCD, despite that in most cases, CCDs are accompanied by other cardiac or extracardiac manifestations. In our study, none of the patients with variants in *LMNA* and *EMD* had DCM or myopathy. Some of the *LMNA* variants identified have been previously described in patients with classic DCM phenotype; in these cases, CCD probably represents an early marker of the disease and the early identification of the genetic etiology is of utmost importance, because it has been recommended that patients with CCD and *LMNA* variants should receive an implantable cardiac defibrillator rather than conventional PPM based on the high incidence of ventricular arrhythmias and SCD that these patients exhibit.[19,20]

All major sarcomeric genes were included for evaluation in our study, despite evidence that the association with CCD is not strong for all of them. Yet, variants in sarcomeric genes were more prevalent in patients with CCD than in control subjects. The association was strong for certain genes such as *MYH7* and *ACTC1*, where variants in patients with CCD were clearly enriched. In addition, sarcomeric genes represented the second largest group of genes among patients with actionable variants (3 in *MYH7*, 2 in *MYBPC3*, and 1 in *ACTC1*). As with the inner nuclear membrane genes, none of these individuals had signs of overt cardiomyopathy at the time of PPM implantation. Interestingly, the 3 patients initially excluded because they had LVMWT ≥15 mm were also genotyped and exhibited pathogenic variants in *MYH7*, *MYBPC3,* and *TRIM63* (homozygous carrier). Altogether, these results suggest that genetic variants in sarcomeric genes might be more frequent than previously thought in patients with CCD even in the absence of overt structural heart disease, in a form that resembles the very recent important description of increased prevalence of disease-causing variants in cardiomyopathy-associated genes among autopsy-inconclusive SCD cases (concealed cardiomyopathy).[21] Additional studies should confirm our results and investigate if CCDs are involved in the pathogenesis of SCD in patients with concealed cardiomyopathy.

In contrast, although fibrosis infiltration of the His bundle has been described in half of SCD cases with a diagnosis of arrhythmogenic right ventricular cardiomyopathy,[22] and CCD has been described as the first manifestation of arrhythmogenic right ventricular cardiomyopathy,[23] we did not find enrichment of very-rare variants in desmosomal genes and did not identify actionable variants in our cohort.

Regarding cardiac channels, variants in these genes were enriched in CCD probands compared with control subjects. This is not surprising, because several of these genes have been related to CCD, even though the evidence is solid only for a limited number. Among them, *SCN5A* is probably the most relevant gene, and in fact, 2 actionable variants in this gene were detected in our study, confirming its importance. Of the remaining, a disease-causing variant in *TRPM4* was identified in 1 proband, and variants in *KCNJ2* were significantly enriched. Interestingly, rare variants in *HCN4*, a gene that was associated with bradycardia/sick sinus syndrome and left ventricular noncompaction, were not enriched (and no actionable variants were detected). Possible explanations for this finding are that the functional impact of disease-causing variants in *HCN4* could be low with presentation confined to asymptomatic bradycardia not requiring PPM,[24] or that the limited number of patients included in our study did not allow to detect significant differences. Of note, variants in other cardiac channel genes, such as *SCN4B*, *CACNA1C,* and *CACNA1D*, were also not significantly enriched.

On the contrary, among regulatory genes (transcription factors and phosphorylating proteins), variants in *TNNI3K* were significantly enriched and an actionable variant with evidence of cosegregation in 1 family was identified, confirming its importance in CCD pathogenesis as previously suggested.[25]

Last, we did not find candidate variants in other genes like *GLA, LAMP2,* and *PRKAG2,* even though CCD is considered a common feature in Fabry disease and glycogen storage cardiomyopathies.[26-28] Two potential explanations for this finding are that these diseases might be too rare and, therefore, do not contribute substantially to CCD globally, or that in these diseases, CCD requiring PPM mostly appears once the cardiomyopathy is stablished.

STUDY LIMITATIONS. There may have been a referral bias because most participating centers were high-complexity centers with specialized units for inherited cardiac diseases and cardiac arrhythmias. This could limit the generalizability of our findings to other patient populations. Although all patients underwent whole exome sequencing, we restricted our analysis to cardiovascular genes with evidence of association with CCD. Accordingly, we cannot exclude that the prevalence of actionable variants in young individuals with CCD found in our study is underestimated because of causal variants in genes that were not analyzed in our study. Nevertheless, we believe that variants in other genes are unlikely to be

classified as pathogenic or likely pathogenic without additional evidence such as family segregation or functional studies.

The definition of unexplained CCD did not include a normal cardiac magnetic resonance imaging. Therefore, we cannot fully exclude that a proportion of patients included were affected by other diseases known to cause CCD, such as cardiac sarcoidosis. In the same line, we cannot discount that in some of the patients with SND, the PPM was implanted because of vagally induced extreme bradycardia or sinus pauses. Also, patients with CCD included in the study might develop cardiomyopathy with extended follow-up.

Finally, the retrospective nature of our study should be taken into consideration, and our results must be replicated, ideally in a large prospective study.

CONCLUSIONS

Our study highlights the importance of genetic testing in patients age ≤60 years with CCD requiring PPM implantation. It demonstrates a higher prevalence of rare variants compared with control subjects, and also that a high proportion of patients with CCD have disease-causing variants that are actionable in clinic, with possible therapeutic consequences for these patients and their families. Moreover, the identification of a genetic etiology in patients with CCD without structural heart disease opens new opportunities to apply tailored genetic therapies that are currently under investigation.[29] Based on our findings, we believe that genetic testing should be offered to patients age ≤60 years with CCD of unexplained origin, allowing for a better risk stratification and management of patients and family members.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Rare protein altering genetic variants in CCD-associated genes are more frequent in patients age ≤60 years with CCD requiring PPM implantation than in the general population.

COMPETENCY IN PATIENT CARE: Genetic testing should be offered to young adults with CCD of unknown cause requiring PPM, allowing a better risk stratification and management of patients and family members.

TRANSLATIONAL OUTLOOK 1: Further studies are needed to understand how family history and genetic testing can be used to identify patients at risk of developing CCD, and how they can be effectively employed to reduce complications associated with CCD including SCD.

TRANSLATIONAL OUTLOOK 2: Larger studies of patients who experience SCD without overt structural disease harboring variants in sarcomeric genes and in their relatives may clarify the role of structural genes in CCD pathogenesis, informing prognosis and improving diagnosis and management strategies.

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KEY WORDS cardiac conduction disorders, cardiomyopathy, genetics, pacemaker, sudden cardiac death

APPENDIX For an expanded Methods section as well as supplemental tables, please see the online version of this paper.