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CLINICAL RESEARCH

Phenotypic expression of rare progressive cardiac conduction disease variants in the general population

Ravi A. Shah [1], Julia Ramírez [2,3,4], Claire Kirkby [5], Charlotte Ives[5], Martin Lowe[5], Patricia B. Munroe [2], Pier D. Lambiase [1,5]*†, and William J. Young [2,5]*†

[1]Institute of Cardiovascular Science, University College London, Rayne Building, University Street, London WC1E 6BT, UK; [2]Centre for Clinical Pharmacology and Precision Medicine, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK; [3]Aragon Institute of Engineering Research, University of Zaragoza, Zaragoza, Spain; [4]Centro de Investigación Biomédica en Red–Bioingeniería, Biomateriales y Nanomedicina, Zaragoza, Spain; and [5]Barts Heart Centre, St Bartholomew's Hospital, W Smithfield, London EC1A 7BE, UK

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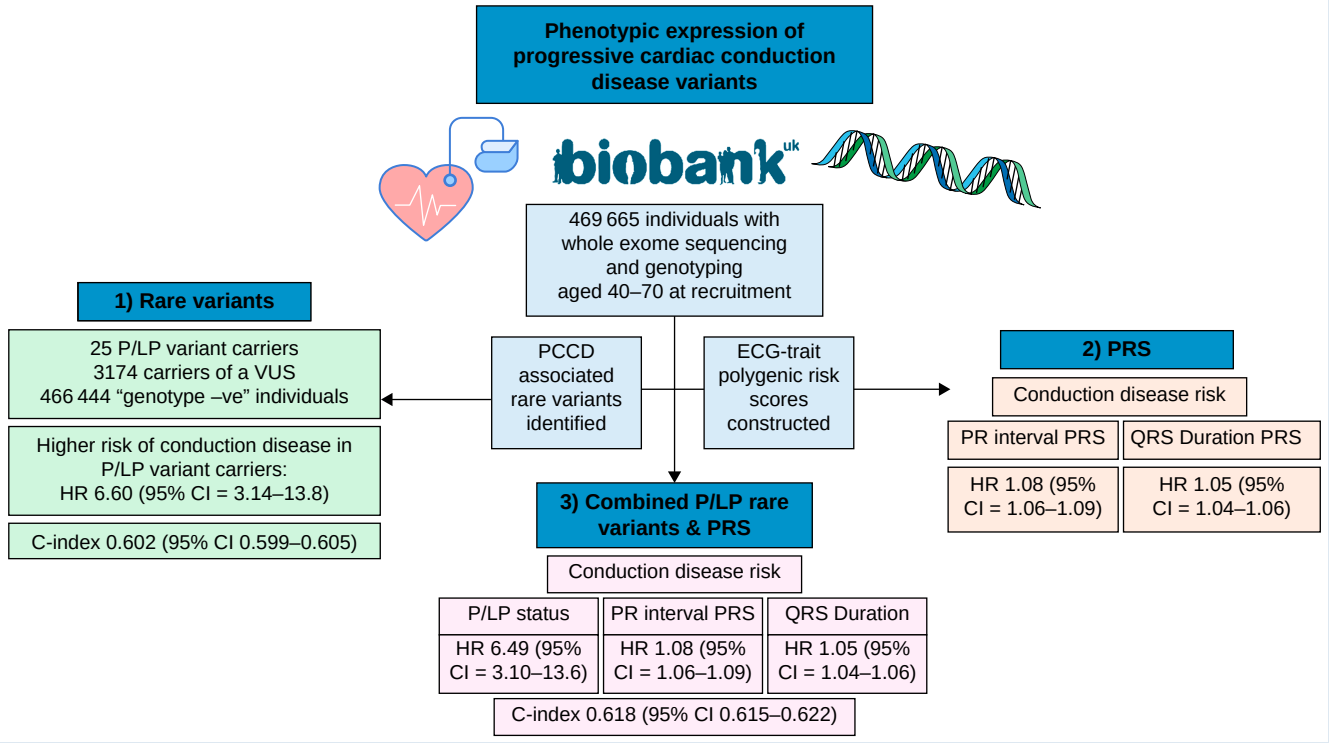
Aims Familial progressive cardiac conduction disease (PCCD) is a heritable condition leading to conduction defects that may require pacemaker implantation. The penetrance of rare PCCD variants in general populations and relationship with electrocardiogram (ECG) trait polygenic risk scores (PRS) is unknown. We investigated the prevalence and phenotypic expression of rare variants linked with PCCD in a population cohort and to establish whether ECG-trait PRSs improve risk prediction.

Methods and results Carriers of known rare pathogenic/likely pathogenic (P/LP) PCCD variants, and variants of uncertain significance (VUS) were identified in 469 511 UK Biobank participants. Primary (any conduction disease) and secondary (high-grade AV block and pacemaker implantation) outcomes were evaluated in lifetime-risk Cox proportional hazard models including rare variant status, sex, and age. Additional models including PR and QRS PRSs were tested. There were 25 P/LP carriers (5 genes) and 3174 VUS carriers (4 genes). Conduction disease was more prevalent in P/LP individuals compared with non-carriers (28% vs. 5.3%, $P < 0.001$) with a hazard ratio (HR) of 6.60 (95% CI = 3.14–13.8) over 6.5 million person-years of follow-up and C-index 0.602 (0.599–0.605). This was driven by AV block (HR 23.2 [8.7–61.8]) and pacemaker implantation (HR 13.4 [6.01–29.8]). All individuals were aged >50 at diagnosis. Combined with P/LP status, PR-PRS and QRS-PRS improved model performance (C-index 0.618 [0.615–0.622]).

Conclusion In a population-based cohort, PCCD P/LP variant carriers were at greater risk of conduction disease. Including PRSs for the PR and QRS improved risk prediction, supporting the combination of rare and common variants in risk assessment.

* Corresponding authors. Tel: +020 7882 5555. *E-mail address*: w.young@qmul.ac.uk (W.J.Y.); Tel: +020 7679 2000. *E-mail address*: p.lambiase@ucl.ac.uk (P.D.L.)
† Jointly supervised this study.

Graphical Abstract



Keywords Atrioventricular block • Penetrance • Genetic risk score • UK Biobank • Inherited cardiovascular disease

What's new?

- Familial progressive cardiac conduction disease (PCCD) is a genetic condition characterised by progressive conduction defects that may lead to pacemaker implantation.
- The prevalence and penetrance of known rare PCCD associated variants in a general population, and their relationship with ECG trait polygenic risk scores is unknown.
- This was investigated using the UK Biobank.
- We have found that rare PCCD variant carriers have a 6.5-fold increased lifetime risk of developing cardiac conduction disease.
- Combining ECG-trait PRSs with rare variant status increased conduction disease risk prediction accuracy.
- These findings support combining rare and common variants in conduction disease risk assessment.

Introduction

Cardiac conduction disease is a heterogenous condition characterised by impaired cardiac electrical signal conduction that can manifest as QRS duration prolongation, bundle branch block or atrioventricular block on the electrocardiogram (ECG).[1,2] Individuals may be asymptomatic, or present with dizziness, syncope, or very rarely, sudden death.[1,2] At a population level, the vast majority of conduction disease is secondary to age-related fibrosis of conduction system tissue or ischaemic heart disease.[1,2] However, familial progressive cardiac conduction disease (PCCD) has a genetic basis and typically considered in individuals below 50 years of age that present with ECG changes demonstrating conduction disease, and a relevant family history.[1]

There are two major forms of inherited PCCD, isolated conduction disease and with co-existing structural heart disease. PCCD is a genetically heterogenous condition caused by rare variants within a range of genes that have been identified through familial segregation testing and functional evaluation. These include cardiac ion channel related genes (e.g. *SCN5A* and *TRPM4*), developmental transcription factors (e.g. *NKX2-5* and *TBX5*) and structural proteins (e.g. *GJA5*, *DES*, and *LMNA*). A number of these genes are also associated with other conditions, such as dilated cardiomyopathies, congenital heart disease, and inherited arrhythmias.[1] Causative variants can be pleiotropic, for example, the same *SCN5A* variant can be associated with both Brugada syndrome and PCCD.[3] Current guidelines recommend genetic testing for PCCD only in patients under 50 years old at presentation.[2]

Most of our understanding of PCCD has been derived from phenotype-first approach to identify causative variants in individuals with suspected progressive familial heart block.[1] This has been essential in the classification of variants, identification of candidate genes, and the understanding of molecular pathophysiology. Ochoa *et al.*[4] recently identified a greater prevalence of rare CCD-related variants in patients with conduction disease of uncertain aetiology who were <60 years old compared with controls. Indeed 14% of probands harboured a variant considered to be actionable.[4] However, the prevalence of known PCCD associated variants in a population cohort and the lifetime risk of developing cardiac conduction disease in carriers is unknown. This limits our interpretation of findings from genetic testing and ability to adequately risk stratify in the inherited cardiac conditions clinic. It is also unknown whether common or low-frequency genetic variation influence the phenotypic expression of PCCD variants, despite previous observations that ECG trait polygenic risk scores are associated with

distal cardiac conduction disease and pacemaker implantation.[5,6] Previous studies have provided support for this in rare genetic diseases, including Brugada and Long QT syndrome.[7,8]

In this study, we have utilised the UK Biobank, a large population level cohort, to investigate: (i) the prevalence of known PCCD pathogenic variants and variants of uncertain significance (VUS) in the general population; (ii) the lifetime risk of developing distal conduction disease phenotypes in PCCD variant carriers; (iii) whether ECG trait common variant polygenic risk scores (PRS) improve risk prediction in rare variant carriers.

Methods

Study population

The UK Biobank is a prospective cohort study comprising around 500 000 individuals. These individuals were recruited from 22 assessment centres across the UK and were aged between 40 and 69 years at recruitment.[9] They answered extensive health and lifestyle questions to provide baseline comorbidity information. They underwent physical measurements and provided biological samples which were used for genotyping by imputation and whole exome sequencing (WES). UK Biobank participants are linked to their hospital inpatient admissions, providing International Classification of Diseases, Tenth Revision (ICD-10) diagnostic and OPCS-4 operation codes, as well as dates of diagnosis. Individuals are also linked to national death registries giving information on date and cause of death. Data from inpatient admissions and death registries was updated until 17th December 2022. The UK Biobank has ethical approval from the North West Multi-centre Research Ethics Committee granted initially in 2011. Its most recent renewal was in 2021, lasting until 2026 (21/NW/0157). Participants (469 665 individuals) who had undergone WES were used in this study. Individuals who have withdrawn consent were removed. This study was performed under UK Biobank application 8256. The reporting of this cohort study follows the Strengthening the Reporting of Observational Studies in Epidemiology statement.

PCCD rare variant selection and annotation

Rare variants known to be associated with PCCD were identified through three sources (see Supplementary material online, *Table S1*). This included variants associated with isolated PCCD, as well as variants associated with PCCD and overlapping syndromes (e.g. cardiomyopathies or Brugada). Variants solely associated with other inherited cardiac conditions but not PCCD were not included. Firstly, VUS, likely pathogenic (LP), and pathogenic (*P*) variants listed in ClinVar and associated with 'heart block' or 'atrioventricular block' phenotypes were included. Secondly, a PubMed literature search was performed using a combination of 'heart block', 'atrioventricular block', and 'conduction disease' terms with 'variant' or 'mutation'. This identified additional PCCD variants not yet captured in the ClinVar data but had support from case series and reporting from other hospital institutions. For these additional variants that had not been formally classified, they were annotated with Ensembl variant effect predictor[10] and classified according to American College of Medical Genetics and Genomics (ACMG) criteria[11] and included in the study if P/LP or a VUS. Thirdly, variants were also identified from the genetic testing database at St Bartholomew's Hospital, for patients undergoing testing for early-onset conduction disease.

All identified pathogenic/likely pathogenic variants underwent manual validation using ACMG criteria.[11] There were no ClinVar variants with conflicting classifications of pathogenicity where one of the conflicts was a pathogenic or likely pathogenic interpretation. VUS and benign/likely benign conflicting interpretations were downgraded to benign/likely benign. As the prevalence of PCCD is unknown, there is no consensus on an allele frequency threshold to report variants with potential to cause disease in isolation. Therefore, when classifying variants against ACMG criteria, an allele frequency of >0.0001 (0.01%), was used to report variants with a frequent greater than expected for the disorder. The same allele frequency is used in Long QT syndrome.[12]

Identification of PCCD rare variant carriers in UKB

The WES protocol for UKB participants has previously been described by Backman *et al.*[13] PCCD variant carriers were identified from project-level variant call format files (field ID 23157) using bcftools v1.15.1. Individuals that failed genetic quality control (call quality ≥ 20, read depth ≥ 10, genotype quality ≥ 20) were removed. Individuals that were carriers of at least one variant formed the genotype-positive (G+) group, and those without any variants formed the genotype-negative (G−) group. The genotype-positive group was further split into those with a P/LP variant or a VUS.

Phenotype definitions

Phenotype definitions were based on ICD-10 (International Classification of Diseases, Tenth Revision) diagnostic codes, OPCS-4 (the Office of Population Censuses and Surveys Classification of Interventions and Procedures version 4) operation codes, and death registry information. A full list of definitions is available in Supplementary material online, *Table S2*. Briefly, conduction disease was defined as any of 1st/2nd/3rd atrioventricular block, fascicular block, bundle branch block (pooled left and right bundle branch block), or pacemaker implantation. To identify individuals with a class I conduction disease indication for a permanent pacemaker, a separate 'high-grade atrioventricular block' definition (2nd or 3rd degree atrioventricular block) was also used.[2] Other important comorbidities included atrial fibrillation, diabetes, hypertension, heart failure, ischaemic heart disease, and dilated cardiomyopathy (full definitions in Supplementary material online, *Table S2*). As the UK Biobank provides access to inpatient hospital admission data that predates the enrolment of the individuals within the biobank, individuals with pre-existing conduction disease were not censored for the study.

Polygenic risk scores

To test the role of common variation in PCCD rare variant phenotypic expression, PR interval and QRS duration PRSs were constructed. These ECG traits were selected due to their known associations at a population level, with cardiac conduction disease.[5,6] As the largest PR and QRS genome-wide association study meta-analyses included UKB Biobank participants, variant effect sizes were recalculated having excluded UKB summary statistics to prevent sample overlap.

Imputed genotype probability data was extracted for each previously reported PR and QRS variant and a 0.9 hard-call threshold applied by coding dosage data as 0, 1, or 2 depending on the number of copies of the effect allele (aligned to the trait increasing allele). Imputed probabilities outside the 0.9 probability threshold were coded as missing. ECG trait specific PRSs were then constructed using an additive model with PRSice-2 v2[14] by summing the number of effect alleles weighted by their betas (milliseconds) aligned to the trait increasing allele. Missing genotypes were imputed with the minor allele frequency within the UK Biobank as is default in PRSice-2. PRSs were standardized to a population mean of 0 and standard deviation of 1 to facilitate effect size comparisons across PRSs.

Statistical analysis

Statistical analysis was performed in R 4.3.2[15] using tidyverse 2.0.0, survival 3.5-7, and tableone 0.13.2 packages.

Baseline characteristics and phenotypes between the P/LP, VUS, and G− group were compared. Continuous, normally distributed variables were summarised using mean and standard deviation and compared across all three groups with ANOVA (analysis of variance). Categorical data was compared across the three groups with the chi-squared test.

Time-to-event analysis was performed using Cox proportional hazard regression to calculate lifetime risk. The data was treated as left truncated (by date of enrolment) and right censored. Age was used as a timescale rather than time-on-study to reduce bias.[16] The model was controlled for sex. The primary outcome was development of any conduction disease. Secondary outcomes were development of: (i) bundle branch block, (ii) high-grade atrioventricular block, or (iii) pacemaker implantation. All individuals who passed genetic quality control were included.

These regression analyses were repeated including sex, each ECG PRS, the first genetic principal components and genotyping array as covariates in the Cox model. PRS hazard ratios are reported per unit of standard deviation (SD) increase. The C-index of each Cox model was calculated and their standard errors were used to determine a 95% confidence interval to compare different model performance.

Sensitivity analyses were performed. Firstly, related individuals were excluded (defined by kinship coefficient < 0.0883) to reduce bias related to shared genetic variation. Secondly, the analysis was performed in individuals of white European ancestry only, as variants may be correlated with ancestry. Ancestry was genetically determined by *k*-means clustering, as used previously.[6] Thirdly, the Cox proportional hazard models were adjusted for co-existing ischaemic heart disease and heart failure at recruitment, as these are known to be associated with cardiac conduction disease, by inclusion of these variables as covariates. Fourthly, further adjustments were made for hypertension, diabetes, smoking status, hypercholesterolaemia, in addition to heart failure and ischaemic heart disease, by including these also as covariates in the Cox proportional hazard models. Fifthly, PRS analysis was additionally completed in a subset of the population who had not been included in the PR and QRS GWAS meta-analysis.

Results

Identified PCCD-related pathogenic variants and VUSs

Within ClinVar, 692 variants were identified associated with PCCD, of which 18 were P/LP and 674 were VUS. Variants were most commonly identified in *SCN5A*, *TRPM4*, and *TNNI3K* (see Supplementary material online, *Table S1*). Other genes identified included: *LMNA*, *CASQ2*, *TTN*, *MYH7*, *DSP*, *LRRC53*, *DES*, *TET2*, *SYNE1*, *TWNK*, *KCNA5*, *LRRC53* and *AKAP10*. Of the 692 variants, 14 were removed (all VUSs) as their allele frequency was >0.0001. For example, the missense variant *AKAP10*: p.I646V is classified as a VUS in ClinVar, however, in UKB the minor allele frequency was 0.399 indicating it cannot cause disease in isolation, despite being a recognised single nucleotide polymorphism associated with the PR interval.[17] No variants identified in the UK Biobank were VUS/LP conflicting variants.

Literature review identified a further 24 P/LP variants and 8 VUSs, predominantly in *LMNA*, *SCN5A*, and *TRPM4* (see Supplementary material online, *Table S1*). Four additional P/LP variants and 2 VUSs were identified from the patient cohort at St Bartholomew's Hospital (see Supplementary material online, *Table S1*).

In total, 46 P/LP variants, and 683 VUSs were identified across all three sources. Of the 46 P/LP variants, there were 30 missense, 7 indel, 6 splice site, and 3 stop gain variants. Of the 683 VUSs, there were 626 missense, 51 indel, and 6 microsatellite variants.

Study population and baseline characteristics

Of the 469 665 UK Biobank individuals with WES available, 469 513 participants passed genetic quality control (*Figure 1*).

In total, 25 UKB participants were carriers of a P/LP variant and 3174 were carriers of at least one VUS. Thirty-eight UKB individuals were carriers of 2 VUSs. The 466 444 remaining individuals were classified as 'genotype-negative' (G−) (*Table 1*). Of the P/LP carriers, there were 7 unique variants in 5 genes (*LMNA*, *SCN5A*, *TNNI3K*, *TRPM4*, and *TTN*). Of the VUS carriers, there were 242 unique VUSs in 4 genes (*MYH7*, *SCN5A*, *TNNI3K*, and *TRPM4*) (see Supplementary material online, *Table S3*).

The three groups had similar mean ages at recruitment (57 years (SD 8.4) in P/LP vs. 56.3 years (SD 8.2) in VUS vs. 56.5 years (SD 8.1) in G−) and at censor date (71.4 years (SD 8.8) in P/LP vs. 70.2 years (SD 8.0) and 70.5 years (SD 8.0) in G−). The percentage of females was similar between each group (60% of P/LP were female vs. 54.3% VUS vs. 54.2% in G−). There was no difference in the prevalence of ischaemic heart disease (6.5–8.0%) between groups but P/LP were more likely to have a diagnosis of heart failure compared with VUS and G− (16% vs. 3.8% vs. 4.2%, $P = 0.008$).

Pathogenic/likely pathogenic variant carriers

Amongst the 25 P/LP variant carriers, 7 different variants were identified affecting *LMNA* (missense; c.949G > A [p.E317K]), *SCN5A* (splice site donor missense; c.3840 + 1G > A), *TNNI3K* (missense; c.2302G > A [p.E768K]), *TRPM4* (two different missense; c.1127T > C [p.p.Ile376Thr] and c.2741A > G [p.Lys914Arg]), and *TTN* (2 different premature stop codons; c.57331C > T and c.82240C > T). Minor allele frequencies ranged between 0.0001 and 0.0006%. Two individuals were related (kinship coefficient = 0.28) and were carriers of a *TTN*: p.R10238* variant. Seven (28%) individuals had conduction disease, of which 6 had pacemakers, and 5 had atrioventricular block (one individual had a diagnostic code for pacemaker implantation but not atrioventricular block, *Table 1*). *LMNA* E317K was responsible for most of the penetrant disease, with 4 out of 6 carriers demonstrating atrioventricular block and had pacemaker implantation. *SCN5A* c.3840 + 1G > A was found in 4 individuals of which only 1 had a pacemaker. *TNNI3K* E768K was carried by 6 individuals, of which 2 had a diagnosis of conduction disease, and carriers of the remaining variants (*TRPM4* and *TTN*) did not have a diagnosis of conduction disease. These findings indicate a degree of variable disease penetrance. All individuals received their diagnosis of conduction disease after the age of 50, at an average of 69 years of age. No individuals had an ICD implanted. Only one individual had a formal diagnosis of dilated cardiomyopathy, a *TNNI3K* E768K variant carrier. This individual did not have a conduction disease phenotype. Two individuals had a diagnosis of both heart failure and conduction disease (both *LMNA* E317K carriers). One of these developed conduction disease 10 years before developing heart failure, and the second received both diagnoses on the same date. A diagnosis of atrial fibrillation was present in 5 out of 7 individuals with penetrant conduction disease and only in 1 out of 18 individuals without penetrant disease (see Supplementary material online, *Table S4*).

Effect of PCCD associated variants on risk for conduction disease

P/LP individuals had a higher lifetime prevalence of conduction disease (28% vs. 5.7% [VUS] vs. 5.3% [G−]; $P < 0.001$), which was predominantly driven by atrioventricular block (20% [P/LP] vs. 1.9% [VUS] vs. 1.8% [G−]; $P < 0.001$) and pacemaker insertion (24% [P/LP] vs. 2.6% [VUS] vs. 2.4% [G−]; $P < 0.001$). In P/LP individuals, compared with VUS and G− groups, 1st degree (12% [P/LP] vs. 0.9% [VUS] vs. 1% [G−]; $P < 0.001$), 2nd degree (12% vs. 0.3% vs. 0.3%; $P < 0.001$), and 3rd degree atrioventricular block (8% vs. 0.5% vs. 0.5%; $P < 0.001$) were more common (*Table 1*). Broadly, the prevalence of conduction disease in VUS carriers was similar to the G− group.

Over 6 547 757 person-years of follow-up, only 1178 G− and 12 VUS individuals (and no P/LP) were lost to follow-up. Cox proportional hazard regression showed P/LP participants had over 6 times increased lifetime risk of developing conduction disease as compared to G− (HR 6.60 [95% CI = 3.14–13.8]) (*Figure 2A*). The VUS group did not have a statistically significant increased lifetime risk of conduction disease (HR 1.11 [95% CI = 0.958–1.28]).

This increased risk of conduction disease in P/LP individuals was driven by an increased lifetime risk of developing high-grade atrioventricular block (HR 23.2 [95% CI = 8.7–61.8]) and were therefore also at greater risk for requiring pacemaker implantation (HR 13.4 [95% CI = 6.01–29.8]). VUS participants did not have increased lifetime risk of reaching these specific secondary endpoints compared with G− individuals with a hazard ratio of 1.02 (95% CI = 0.686–1.53) and 1.16 (95% CI = 0.933–1.43) for high-grade atrioventricular block and pacemaker insertion respectively (*Figure 2B* and *C*). None of the P/LP individuals had bundle branch block, and therefore this secondary outcomes could not be assessed.

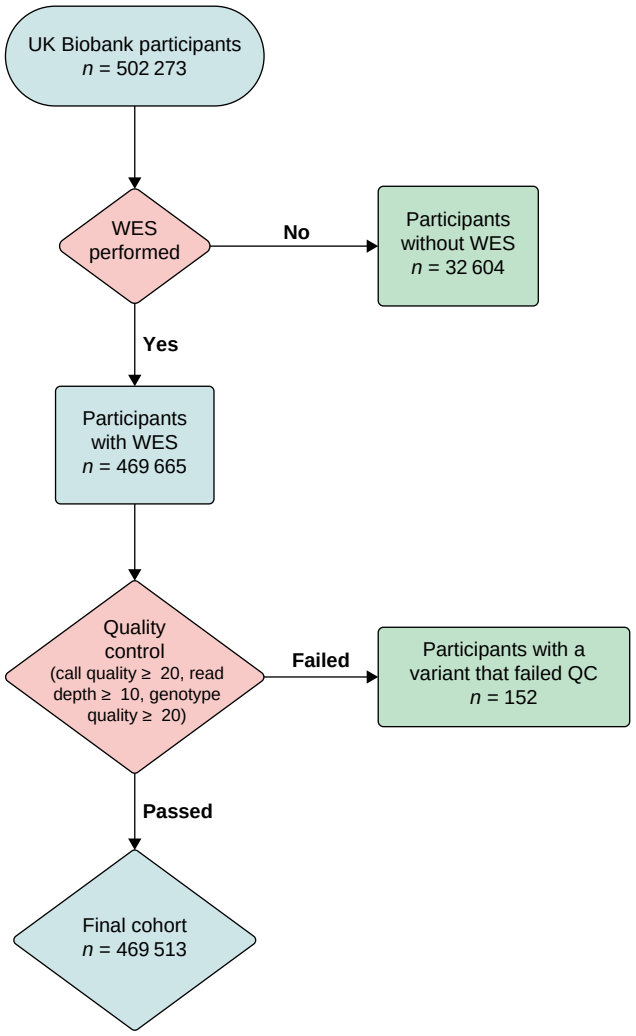


Figure 1 Cohort selection process. QC, quality control; WES, whole exome sequencing.

Sensitivity analyses

In an unrelated subset (434 985 participants) and in individuals of white European ancestry only, population lifetime risk of developing conduction disease, high-grade atrioventricular block, and requiring pacemaker status was significantly greater in the P/LP group with similar hazard ratios to the main model (see Supplementary material online, *Tables S5–S7*). When controlling for ischaemic heart disease and heart failure, hazard ratios were significant, but marginally reduced compared with the main model (see Supplementary material online, *Tables S5–S7*). Hazard ratios remained significant when adjusting for hypertension, diabetes, smoking status, hypercholesterolaemia, smoking status, heart failure, and ischaemic heart disease (see Supplementary material online, *Tables S5–S7*). Ten-year risk after enrolment of developing conduction disease, high-grade atrioventricular block, and requiring pacemaker implantation showed similar results (see Supplementary material online, *Table S8*).

The effect of PR interval and QRS duration PRSs on developing conduction disease

The PR PRS was associated with conduction disease (HR = 1.08 [95% CI = 1.06–1.09]), high-grade atrioventricular block (HR = 1.10 [95% CI = 1.06–1.13]) and pacemaker implantation (HR = 1.04 [95% CI = 1.02–1.06]) in UKB individuals (*Table 2*). The QRS duration PRS was predictive of the broad conduction disease definition only (HR = 1.05 [95% CI = 1.04–1.06]) (*Table 3*). This was due to a positive association between bundle branch block and QRS PRS (HR = 1.10 [95% CI = 1.08–1.12]).

In a combined Cox regression model with both rare variant status and PRSs, P/LP status, PR interval PRS, and QRS duration PRS were independent predictors of developing conduction disease with a hazard ratio of HR = 6.49 (95% CI = 3.1–13.6), HR = 1.08 (95% CI = 1.06–1.09), and HR = 1.05 (95% CI = 1.04–1.06) respectively (*Table 4*). The hazard ratios in the combined PR interval PRS and rare variant models are similar to those of the individual models, suggesting the PRS is capturing an independent risk element as compared to the rare variants. The C-index of the model including rare variant status, PR-PRS, and QRS-PRS was higher than the model including rare variant status only (0.618 [95% CI 0.615–0.622] vs. 0.602 [95% CI 0.599–0.605]).

Next, we assessed whether the PRS can predict disease specifically within participants carrying VUS. The PR PRS, but not QRS PRS, was associated with increased risk of high-grade atrioventricular block (HR = 1.55 [95% CI = 1.03–2.34]) in the VUS group (*Table 5*), with a model C-index of 0.793 (95% CI 0.704–0.881). In comparison, within

Table 1 Summary of demographics and clinical variables

	G–	Uncertain significance	Pathogenic/likely pathogenic	*P* value
n	466 444	3174	25	
Age (years)	70.51 (8.03)	70.15 (8.03)	71.44 (8.76)	0.039
Age at recruitment (years)	56.54 (8.09)	56.27 (8.16)	57.00 (8.41)	0.149
Female (%)	252 805 (54.2)	1722 (54.3)	15 (60.0)	0.843
Genetic ancestry (%)				<0.001
European	441 595 (94.7)	2825 (89.0)	24 (96.0)	
Asian	10 099 (2.2)	110 (3.5)	1 (4.0)	
African	7411 (1.6)	117 (3.7)	0 (0.0)	
Mixed	5262 (1.1)	79 (2.5)	0 (0.0)	
Chinese	2057 (0.4)	42 (1.3)	0 (0.0)	
Other ethnic group	16 (0.0)	1 (0.0)	0 (0.0)	
Not specified	4 (0.0)	0 (0.0)	0 (0.0)	
Hypertension (%)	150 981 (32.4)	1014 (31.9)	14 (56.0)	0.036
Diabetes (%)	43 327 (9.3)	296 (9.3)	5 (20.0)	0.182
Ischaemic heart disease (%)	33 254 (7.1)	206 (6.5)	2 (8.0)	0.373
Heart failure (%)	19 362 (4.2)	122 (3.8)	4 (16.0)	0.008
Atrial fibrillation (%)	38 475 (8.2)	267 (8.4)	6 (24.0)	0.016
Atrioventricular block (%)	8355 (1.8)	60 (1.9)	5 (20.0)	<0.001
1st degree atrioventricular block (%)	4621 (1.0)	30 (0.9)	3 (12.0)	<0.001
2nd degree atrioventricular block (%)	1611 (0.3)	9 (0.3)	3 (12.0)	<0.001
3rd degree atrioventricular block (%)	2169 (0.5)	16 (0.5)	2 (8.0)	<0.001
High-grade atrioventricular block (%)	3558 (0.8)	24 (0.8)	4 (16.0)	<0.001
Bundle branch block (%)	11 949 (2.6)	89 (2.8)	0 (0.0)	0.497
Pacemaker (%)	11 052 (2.4)	84 (2.6)	6 (24.0)	<0.001
ICD (%)	1789 (0.4)	15 (0.5)	0 (0.0)	0.687
Fascicular block (%)	307 (0.1)	3 (0.1)	0 (0.0)	0.815
Conduction disease (%)	24 934 (5.3)	182 (5.7)	7 (28.0)	<0.001
Dead (%)	40 895 (8.8)	304 (9.6)	4 (16.0)	0.121
Age at death (years)	71.03 (7.58)	71.56 (6.91)	75.99 (2.59)	0.2

The baseline characteristics of the whole cohort (overall), and the separated groups based on genotype status. Pathogenic/likely pathogenic variant carriers, VUS carriers and genotype-negative (G–) individuals. Continuous variables are summaries as mean (standard deviation) and are compared with ANOVA tests to produce *P* values. Categorical variables are summarised with percentages and compared with Chi-squared test.

the G- group, the PR PRS was associated with a hazard ratio of 1.09 (95% CI 1.06–1.13) for developing high-grade atrioventricular block with a C-index of 0.633 (0.623–0.642) (see Supplementary material online, *Table S9*).

The PRS results remained similar when analysis was reperformed in the cohort subset (333 972 individuals) who were not included in the original PR and QRS GWAS meta-analysis study (see Supplementary material online, *Tables S10*–S12).

Discussion

Using the UK biobank (a large prospective population-based cohort), we have investigated the prevalence, penetrance, and expressivity of known variants associated with PCCD and tested the effects of combining them with common variation in a conduction disease prediction model. We have several important findings. Firstly, P/LP rare variant carrier status predicts a 6.5-fold increased lifetime risk of cardiac conduction disease, a 23-fold increased lifetime risk of atrioventricular block, and 13-fold increased lifetime risk of pacemaker requirement. Secondly, we have demonstrated that the phenotypic expression of some P/LP variants is highly variable. Thirdly, a PR interval and QRS duration PRS captures risk of developing conduction disease that is independent of P/LP variants and may improve risk prediction in carriers of a VUS.

Risk prediction associated with rare variants

The relationship of rare PCCD associated variants with risk for conduction disease is poorly understood, impacting the ability to make informed decisions on risk stratification and recommendations for device implantation to preventing life-threatening bradyarrhythmia.[1] Using a population-based study with participants recruited between the ages of 40–69 years, we have observed a significant increased lifetime risk for conduction disease, including high grade AV block in P/LP PCCD variant carriers. This is a novel finding and was also

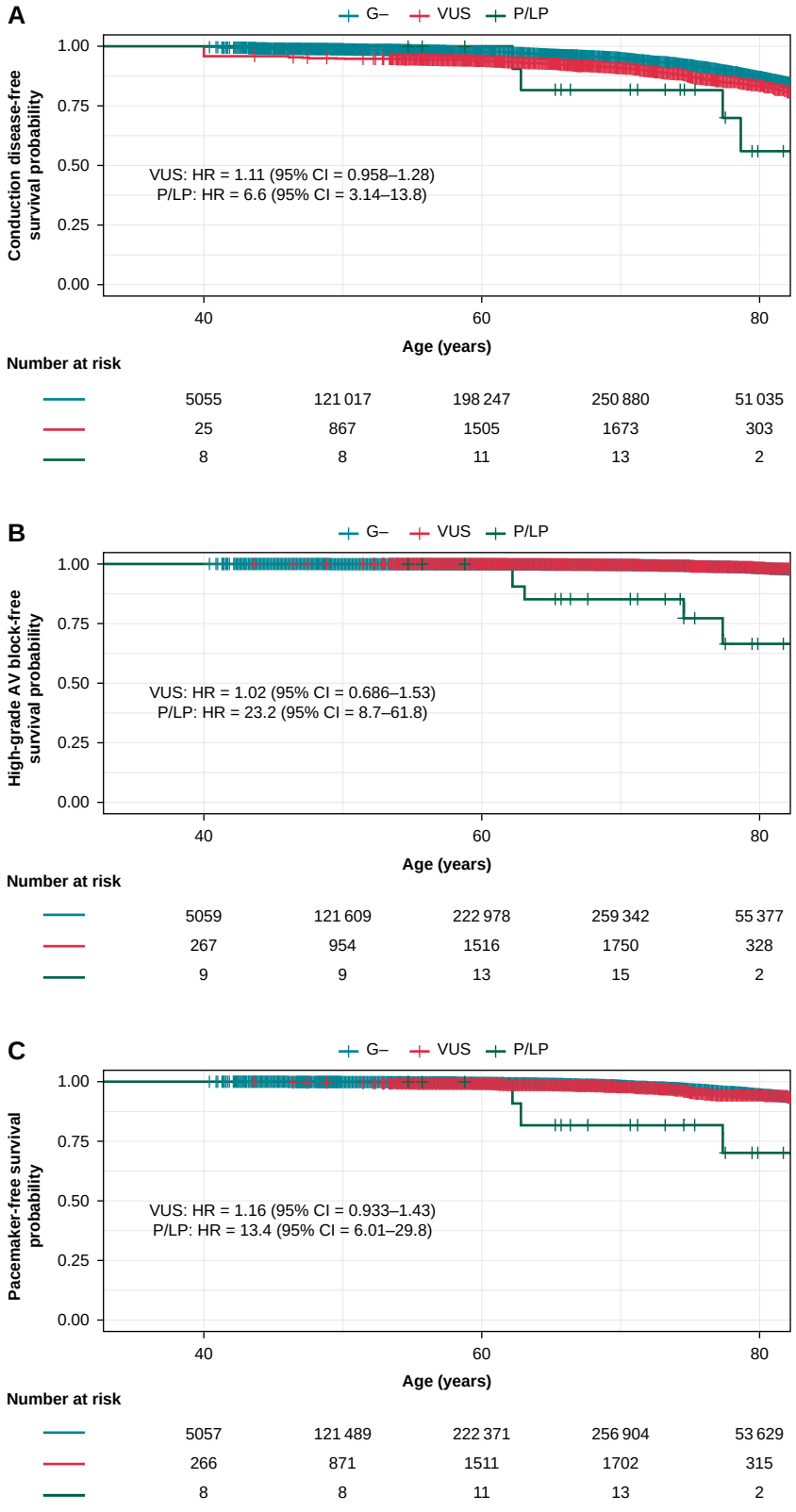


Figure 2 Kaplan-Meier plots showing the risk of developing the primary and secondary outcomes. Kaplan-Meier plots show the risk of developing conduction disease (*A*), atrioventricular block (*B*), and pacemaker implantation (*C*) as stratified by genotype status. Hazard ratios and 95% confidence intervals are calculated by Cox proportional hazard regression corrected for sex and using age as the timescale. Hazard ratios are in comparison to the genotype-negative group. G–, genotype-negative; VUS, variants of uncertain significance; P/LP, pathogenic/likely pathogenic variants; HR, hazard ratio; CI, confidence interval.

Table 2 Cox proportional hazard models for risk of developing conduction disease, high grade atrioventricular block, or requiring pacemaker implantation as predicted by the PR interval PRS controlling for sex, the first 10 genetic principal components, genotyping array, and using age as a timescale in the entire cohort (including rare variant carriers)

Outcome	Risk factor	Hazard ratio
Conduction disease	PR PRS	HR = 1.08 (95% CI = 1.06–1.09)
	Male	HR = 2.25 (95% CI = 2.20–2.31)
High-grade atrioventricular block	PR PRS	HR = 1.10 (95% CI = 1.06–1.13)
	Male	HR = 2.54 (95% CI = 2.37–2.73)
Pacemaker implantation	PR PRS	HR = 1.04 (95% CI = 1.02–1.06)
	Male	HR = 2.59 (95% CI = 2.49–2.70)

For each outcome the hazard ratio associated with the listed risk factor in multivariable analysis is given.

Table 3 Cox proportional hazard models for risk of developing conduction disease, high grade atrioventricular block, or requiring pacemaker implantation as predicted by the QRS duration PRS controlling for sex, the first 10 genetic principal components, genotyping array, and using age as a timescale, in the entire cohort (including rare variant carriers)

Outcome	Risk factor	Hazard ratio
Conduction disease	QRS PRS	HR = 1.05 (95% CI = 1.04–1.06)
	Male	HR = 2.26 (95% CI = 2.20–2.31)
High-grade atrioventricular block	QRS PRS	HR = 1.03 (95% CI = 0.996–1.06)
	Male	HR = 2.55 (95% CI = 2.37–2.73)
Pacemaker implantation	QRS PRS	HR = 1.02 (95% CI = 0.999–1.04)
	Male	HR = 2.59 (95% CI = 2.49–2.70)

For each outcome the hazard ratio associated with the listed risk factor in multivariable analysis is given.

significant in sensitivity analyses. These results build on the recent findings by Ochoa *et al.*[4] demonstrating a higher prevalence of rare variants in conduction disease associated genes in patients with pacemaker implantation below the age of 60 compared with controls.[4] In addition to using a population-based cohort, our prospective study enabled assessment of lifetime risk.

Surprisingly, all P/LP variant carriers with conduction disease did not receive this diagnosis until older than 50 years of age and therefore in line with current international guidelines, would not have been offered genetic testing on the basis of ECG findings alone, if seen in the inherited cardiac conditions clinic. While the yield of P/LP variants from genetic testing all middle-aged individuals with conduction disease would be low (as evidenced by this study), knowledge of carrier status could impact choice of device implant and would permit predictive testing in family members facilitating screening strategies for early detection of bradyarrhythmia.

LMNA variant associated with conduction disease

In this study, *LMNA* E317K variant was associated with highly penetrant high grade atrioventricular block requiring pacemaker implantation (4 out of 6 carriers) that developed after 50 years of age and was not associated with dilated cardiomyopathy. This contrasts with the *TRPM4* I376T variant, where no carriers had a conduction disease diagnosis. *LMNA* encodes two proteins lamin A and C (produced by alternative splicing) that are major constituents of the inner nuclear membrane.[1] They have a structural role within the nucleus as well as contributing to gene expression through interactions with chromatin and transcription factors. *LMNA* variants are associated with a wide variety of diseases, including dilated cardiomyopathy, PCCD, atrial and ventricular arrhythmias, and muscular dystrophy.[1] Interestingly, the novel E317K variant in 4 patients reported relatively late-onset of conduction disease (presenting at 44, 64, and 64 years of age) and a long interval till development of dilated cardiomyopathy on echocardiography.[18] Other *LMNA* variants would clinically manifest at an earlier age, highlighting the variable penetrance and expressivity of different variants within the same gene. Our findings indicating that *LMNA* was the gene in which rare variants were most commonly identified in patients with early-onset conduction disease, despite exclusion of individuals with cardiomyopathies were also found in Ochoa *et al.*[4]

Combining common and rare genetic variation in risk assessment

For the first time, we have combined common variation with known rare variants associated with PCCD in the setting of cardiac conduction disease, and assessed their risk in a middle-aged population-based cohort. Although it is known that PR-interval and QRS-duration PRS is associated with lifetime risk of development of cardiac conduction disease and requirement for pacemaker,[5,6] we have demonstrated both these PRS and rare PCCD variants are independently predictive of development of conduction disease and that combining them is more predictive than rare variants alone, as also seen in long QT and Brugada syndrome.[7,8] This suggests that common and rare genetic variation may act through separate non-interacting biological pathways to influence risk for developing cardiac conduction disease. These single nucleotide polymorphisms could potentially explain a subset of the incomplete penetrance and variable expressivity of rare pathogenic variants, although further work with updated PRS construction methods and large numbers of cases would be needed to validate these observations. These findings adds to the growing body of work that rare and common genetic variation both contribute to inherited cardiac conditions.[8]

Clinical relevance

These results are likely to be increasingly relevant for risk stratification in inherited cardiac conditions clinic. There is a growing appreciation of the role of rare genetic variation in cardiac conduction disease.[4,19] Combined with the increasing affordability of next generation sequencing, this suggests that identification of variants in patients will become ever more frequent. It is also likely that identification of asymptomatic variant carriers will become more common, due to cascade screening or incidental findings when sequencing for other conditions. The participants recruited to the UK Biobank study are likely to be representative of such individuals and therefore the significantly increased risks of high-grade atrioventricular block and pacemaker implantation we have found could apply. Understanding these risks are essential to providing accurate information to such patients in the clinic, as well as influencing clinical management, for example, ensuring regular follow-up and ECGs of these at-risk individuals.

In addition, these results taken together with findings from a recent large nationwide cohort study investigating the familial risk for

Table 4 Cox proportional hazard models for risk of developing conduction disease, high grade atrioventricular block, or requiring pacemaker implantation

Outcome	Risk factor	Hazard ratio
Conduction disease	Variants of uncertain significance	HR = 1.10 (95% CI = 0.947–1.27)
	Pathogenic/likely pathogenic	HR = 6.49 (95% CI = 3.10–13.6)
	PR interval PRS	HR = 1.08 (95% CI = 1.06–1.09)
	QRS duration PRS	HR = 1.05 (95% CI = 1.04–1.06)
	Male	HR = 2.26 (95% CI = 2.20–2.31)
High-grade atrioventricular block	Variants of uncertain significance	HR = 1.02 (95% CI = 0.683–1.52)
	Pathogenic/likely pathogenic	HR = 22.4 (95% CI = 8.39–59.7)
	PR interval PRS	HR = 1.1 (95% CI = 1.06–1.13)
	QRS duration PRS	HR = 1.03 (95% CI = 0.996–1.06)
	Male	HR = 2.55 (95% CI = 2.37–2.73)
Pacemaker implantation	Variants of uncertain significance	HR = 1.15 (95% CI = 0.93–1.43)
	Pathogenic/likely pathogenic	HR = 13.1 (95% CI = 5.9–29.2)
	PR interval PRS	HR = 1.04 (95% CI = 1.02–1.06)
	QRS duration PRS	HR = 1.02 (95% CI = 0.999–1.04)
	Male	HR = 2.59 (95% CI = 2.49–2.7)

Cox model covariates were PR interval PRS, QRS duration PRS, and variant carrying status, sex, the first 10 genetic principal components, genotyping array, and controlling for age as a timescale, in the entire cohort. For each outcome the hazard ratio associated with the listed risk factor in multivariable analysis is given.

Table 5 Cox proportional hazard models for risk of developing conduction disease, high grade atrioventricular block, or requiring pacemaker implantation in the variant of uncertain (VUS) significance group as predicted by PR interval and QRS duration PRS and controlling for sex, the first 10 genetic principal components, genotyping array, and using age as a timescale

Outcome	Risk factor	Hazard ratio
Conduction disease	PR PRS	HR = 1.13 (95% CI = 0.970–1.31)
	QRS duration PRS	HR = 1.07 (95% CI = 0.918–1.24)
	Male	HR = 2.21 (95% CI = 1.63–3.01)
High-grade atrioventricular block	PR PRS	HR = 1.55 (95% CI = 1.03–2.34)
	QRS duration PRS	HR = 1.4 (95% CI = 0.911–2.14)
	Male	HR = 3.42 (95% CI = 1.35–8.65)
Pacemaker implantation	PR PRS	HR = 1.08 (95% CI = 0.863–1.34)
	QRS duration PRS	HR = 1.14 (95% CI = 0.913–1.43)
	Male	HR = 2.89 (95% CI = 1.79–4.65)

For each outcome the hazard ratio associated with the listed risk factor in multivariable analysis is given.

pacemaker implantation in sinus node disease, where individuals with an immediate family member with a pacemaker implanted under the age of 60 had a 5.5-fold increased risk in pacemaker requirement,[20,21] suggest that similar approaches may prove informative in other diseases that necessitate permanent pacemaker implantation.

Limitations

As with many large population cohorts, the UK Biobank is affected by healthy volunteer and survival bias, and our findings are reflective of a middle-aged cohort rather than a younger population when PCCD would typically be considered. The majority of participants are of white European ancestry (24/25 P/LP carriers), limiting specific investigation in other ancestries. The number of P/LP PCCD variant carriers in UK Biobank is small; however, this is not unexpected given the objective was to capture rare variation (with MAFs <0.01%) and sensitivity analyses suggest our findings are robust. Additionally, it is highly likely that rare and common genetic variation that influences conduction disease risk is not captured in this study. Specifically, as we have performed curation at a variant level rather than at a gene level, there will be variants with potential PCCD associations that are not included in our study. Such variants could be added to prediction models in the future as additional genetic contributors are identified.[20]

Conclusions

In summary, this study has shown that rare variation has relevance in a middle-aged population with respect to conduction disease risk, and PRSs may provide additional independent information that, when combined with further clinical, metabolic, and environmental factors, could explain a greater proportion of risk and enable early screening strategies for life-threatening bradyarrhythmia.

Supplementary material

Supplementary material is available at *Europace* online.

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Conflict of interest: The authors have no conflicts to disclose.

Data availability

The data underlying this article are available in UK Biobank (www.ukbiobank.ac.uk), which is accessible by approved researchers.

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