SUPPLEMENTAL MATERIAL

Supplemental methods

Definition of PKP2 truncating variants

*PKP2*tv were defined as the consequence of single nucleotide variants that create a premature stop codon or disrupt a splice site; small insertions or deletions leading to a shift in the reading frame; and larger deletions encompassing single or multiple exons (CNVs). Splice variants were defined as canonical if located within the intronic donor and acceptor sites (GT-AG) and first and last two exonic nucleotides, and non-canonical at other intronic locations. As exon 6 is not expressed in the cardiac isoform of *PKP2* (*PKP2A*), variants identified in this exon and associated exon-intron boundaries (12:32996247-12:32996115) were not included.[34] Two frameshift variants at amino acid residues 323 and 325 leading to apparent premature truncation have been shown to cause missense variation so were also not included. [35] All variants were classified using reference sequence GRCh38. Missense variants and in-frame deletions were excluded. Mutant mRNA transcripts where the sequence variant was located 55 base pairs upstream from the terminal 3' exon-exon boundary were considered to undergo nonsense mediated mRNA decay (NMD). [36]

Identification previously reported PKP2tv associated with ARVC

Probands fulfilling Task Force criteria for the diagnosis of ARVC (referred to subsequently as 'ARVC cohort') with associated *PKP2*tv were identified from publications in publicly available medical literature and cited in PubMed related to arrhythmogenic right ventricular cardiomyopathy (ARVC) and truncating variants in *PKP2* (*PKP2*tv). Data was also sourced from a publicly available database, created and hosted by the Leiden University Medical Center (LUMC)[1], and all identified cases cross referenced to the original article. This database is longer available. A targeted literature search was performed up January 2021 to identify suitable publications. The

comprehensive search used the following terms; ((((arrhythmogenic right ventricular [Title]) AND *PKP2*[Title])) OR ((arrhythmogenic right ventricular[Title]) AND plakophilin-2[Title])) OR ((arrhythmogenic[Title] AND cardiomyopathy[ti]) AND plakophilin-2[Title]) ("*PKP2* protein, human"[nm]) AND ("arrhythmogenic right ventricular dysplasia"[MeSH Terms] OR "arrhythmogenic right ventricular dysplasia"[All Fields] OR "arrhythmogenic right ventricular cardiomyopathy"[All Fields]) (((*PKP2*) OR plakophilin 2)) AND ("arrhythmogenic right ventricular dysplasia"[MeSH Terms] OR "arrhythmogenic right ventricular dysplasia"[All Fields] OR "arrhythmogenic right ventricular cardiomyopathy"[All Fields]).

All English language publications identified through the PubMed search were reviewed by two investigators (RJH/DJA) for the number of cases who had a clinical diagnosis of ARVC based on the 2010 Task Force Criteria (or the preceding 1994 Criteria where relevant),[2] associated with a heterozygous *PKP2*tv. To avoid double counting the same proband, publications from the same authors or institutions were cross referenced and only included once unless it was evident that cases were not previously reported based on age, sex and specific *PKP2*tv. Patients with more than one variant in *PKP2* or other desmosomal genes, unexplained cardiac arrest or sudden death and no additional diagnostic characteristics, and those with non-ARVC phenotypes were excluded. Family members were not included. The manuscripts are listed in the supplementary appendix below.

Classification of UK Biobank participants with PKP2tv

Individuals with *PKP2*tv were identified in the UK Biobank (2nd release; October 2020), a large scale, prospective biomedical database with in-depth whole exome sequencing available on 200,643 individuals enrolled between the ages of 40 and 69 at the time of recruitment. Cardiovascular and other clinical data is available for all participants, ascertained through primary care and hospital records, death records and self-reported medical conditions, recorded using ICD-9 or ICD-10 codes, and evidence for specific diagnoses and procedures is included in

the Biobank when identified through any of these sources. Data includes evidence of ischemic heart disease; heart failure; hypertension; atrial and ventricular arrhythmia; surgical and interventional cardiac procedures. Cardiac magnetic resonance (CMR) data included left ventricular ejection fraction (LVEF; %); left ventricular end diastolic function (LVEDV; ml/m^2); and cardiac index (L/m^2). Numerical data derived from 12-lead ECGs data including QRS duration and QT interval corrected for heart rate (QTc) was included, but as actual electrocardiographic traces were not accessible, repolarization patterns and T-wave polarity were not part of the analysis.

Demographic and clinical data was manually curated to identify specific cardiac phenotypes. A diagnosis consistent with ARVC was determined based on cardiovascular features consistent with the condition. To assess the frequency of *PKP2*tv in the absence of manifest clinical disease, individuals with ARVC were removed from further analysis and the remainder without ARVC were used as controls (referred to subsequently as 'UKB cohort').

To determine if *PKP2*tv act as a risk allele for the development of atrial fibrillation (AF), heart failure and ventricular tachycardia (VT) without a diagnosis of ARVC, we assessed the prevalence of these features in those with *PKP2*tv; the proportion of those with AF was compared to all other UK Biobank participants.

Calculation of minor allelic frequency

To compare the prevalence of *PKP2*tv between the UKB cohort and ARVC cohort, the minor allele frequency (MAF) was calculated for all variants identified in both cohorts. For the ARVC cohort comprised of PKP2tv previously reported in the literature, we assumed a disease prevalence of 1 in 5000 in the population,[37] and based on the most comprehensive report to date detailing the

genetic findings in 1001 patients with ARVC that 42.1% of individuals with ARVC was associated with a *PKP2*tv.[4] As such, the population prevalence of ARVC and a *PKP2*tv was ((1/5000)/0.421) = $8.42*10^{-5}$. Each identified *PKP2*tv was e*pressed as a proportion of the overall variant number.

The MAF of each *PKP2*tv was defined by the population prevalence ($8.42*10^{-5}$), the proportion of any *PKP2*tv to the overall burden, and the biallelic nature of individuals; therefore, MAF = ($8.42*10^{-5}$*variant proportion (%))*0.5. For example, Arg413* was identified in 16 ARVC probands and represents 3.27% of the overall contribution of *PKP2*tv to ARVC (16/489). The MAF is therefore calculated as ($8.42*10^{-5}$*0.0327)*0.5 = $1.38 * 10^{-6}$.

The MAF of each variant identified in UKB controls was defined as the number of times each variant was identified relative to the overall number of participants (200,643).

Comparison of allelic frequency between UKB cohort and ARVC cohort

Only *PKP2*tv where the nucleotide substitution, insertion or deletion was identical were included for comparative analysis. The association between *PKP2*tv and clinical ARVC was assessed by comparison of the MAF within each group to define the odds ratio (OR) with 95% confidence intervals for each, where a ratio of >1 would favor a diagnosis of ARVC and a ratio <1 would favor non-penetrant or sub-clinical disease. As CNV were not called in the UK Biobank these were not included in the comparative analysis.

In silico analysis

The predicted deleterious effect of variants in both the ARVC cohort and UKB cohort was assessed using in silico analysis. Premature stop codons and frameshift (insertion/deletions) variants were assessed using combined annotation-dependent depletion (CADD),[38] and splice variants using Splice AI.[39]

Statistical analysis

The odds ratios for the MAF between the ARVC cohort and UKB cohort were calculated with Fisher's exact test (in R) and p-values were Bonferroni corrected to account for multiple testing, with significance level of adjusted to $p < 0.0025$. The OR for prevalence of AF, heart failure and ventricular tachycardia in those with and without *PKP2*tv in UK Biobank was similarly calculated with significance level at <0.05. In silico analyses between respective groups were compared using a two-sided t-test with significance level at <0.05. Statistical analysis was performed using R.

Supplementary table I: 47 PKP2 truncating variants identified in 326 UK Biobank participants.

Transcript Consequence	Consequence	Categorization	N (probands)	Proportion
c.2146-1G>C	.	Splice; acceptor	46	23.8%
c.2203C>T	p.Arg735X	Premature stop codon	40	20.7%
c.148_151delACAG	p.Thr50SerfsX61	Deletion; frameshift	18	9.3%
c.337-2A>T	.	Splice; acceptor	8	4.1%
c.275T>A	p.Leu92X	Premature stop codon	6	3.1%
c.1821dupT	p.Val608CysfsX6	Insertion; frameshift	6	3.1%
c.2489+1G>A	.	Splice; donor	6	3.1%
c.253_256delGAGT	p.Glu85MetfsX26	Deletion; frameshift	5	2.6%
c.2312_2313delTC	p.Leu771ProfsX2	Deletion; frameshift	5	2.6%
c.663C>A	p.Tyr221X	Premature stop codon	4	2.1%
c.358G>T	p.Glu120X	Premature stop codon	4	2.1%
c.1237C>T	p.Arg413X	Premature stop codon	3	1.6%
c.2453dupG	p.Ile819GlyfsX9	Insertion; frameshift	3	1.6%
c.1132C>T	p.Gln378X	Premature stop codon	2	1.0%
c.1709delC	p.Ala570ValfsX7	Deletion; frameshift	2	1.0%
c.1754C>G	p.Ser585X	Premature stop codon	2	1.0%
c.1917_1935dupTGACAAC AACAAAAGTATT	p.Gly646fsX0	Insertion; frameshift	2	1.0%
c.1999G>T	p.Glu667X	Premature stop codon	2	1.0%
c.2299+1G>A	.	Splice; donor	2	1.0%
c.2427delT	p.Asn809LysfsX5	Deletion; frameshift	2	1.0%
c.14delG	p.Gly5AlafsX34	Deletion; frameshift	1	0.5%
c.235C>T	p.Arg79X	Premature stop codon	1	0.5%
c.368G>A	p.Trp123X	Premature stop codon	1	0.5%
c.658C>T	p.Gln220X	Premature stop codon	1	0.5%
c.1063C>T	p.Arg355X	Premature stop codon	1	0.5%
c.1177C>T	p.Gln393X	Premature stop codon	1	0.5%
c.1369_1372delCAAA	p.Gln457X	Premature stop codon	1	0.5%
c.1643delG	p.Gly548ValfsX15	Deletion; frameshift	1	0.5%
c.1689-1G>C	.	Splice; acceptor	1	0.5%
c.2013delC	p.Lys672ArgfsX12	Deletion; frameshift	1	0.5%
c.2198_2202delACACC	p.His733AlafsX8	Deletion; frameshift	1	0.5%
c.2421C>G	p.Tyr807X	Premature stop codon	1	0.5%

c.439A>T	p.Arg147X	Premature stop codon	1	0.5%
c.622_623insAA	p.Thr208LysfsX56	Insertion; frameshift	1	0.5%
c.853dupG	p.Ala285GlyfsX51	Insertion; frameshift	1	0.5%
c.962delT	p.Val321AlafsX37	Deletion; frameshift	1	0.5%
c.971_972insCT	p.Ala325ArgfsX28	Insertion; frameshift	1	0.5%
c.975_976insAGCGGCC	p.Ala326SerfsX12	Insertion; frameshift	1	0.5%
c.1171-1G>C	.	Splice; acceptor	1	0.5%
c.1355T>A	p.Leu452X	Premature stop codon	1	0.5%
c.1521G>A	p.Trp507X	Premature stop codon	1	0.5%
c.1901delA	p.Asn634ThrfsX21	Deletion; frameshift	1	0.5%
c.1905delG	p.Asn636IlefsX20	Deletion; frameshift	1	0.5%
c.2182G>T	p.Glu728X	Premature stop codon	1	0.5%
c.2489+2dupT	.	Splice; donor	1	0.5%

Supplementary table II: 144 PKP2 truncating variants identified in 487 probands with arrhythmogenic right ventricular cardiomyopathy (ARVC cohort).

Transcript Consequence	*Consequence*	*Categorization*	*N (probands)*	*Proportion*
c.2146-1G>C		Splice; acceptor	61	12.53%
c.235C>T	p.Arg79X	Premature stop codon	35	7.19%
c.2489+1G>A		Splice; donor	28	5.75%
c.2197_2202delCACACCinsG	p.His733AlafsX8	Ins/del; frameshift	27	5.54%
c.148_151delACAG	p.Thr50SerfsX61	Deletion; frameshift	25	5.13%
c.1211dupT	p.Val406SerfsX4	Insertion; frameshift	16	3.29%
c.1237C>T	p.Arg413X	Premature stop codon	16	3.29%
c.1613G>A	p.Trp538X	Premature stop codon	12	2.46%
c.2509delA	p.Ser837ValfsX94	Deletion; frameshift	11	2.26%
	Del exon 1-14	CNV	9	1.85%
c.1132C>T	p.Gln378X	Premature stop codon	9	1.85%
c.1643delG	p.Gly548ValfsX15	Deletion; frameshift	9	1.85%
c.1728_1729insGATG	p.Arg577AspfsX5	Insertion; frameshift	9	1.85%
c.1912C>T	p.Gln638X	Premature stop codon	9	1.85%
c.397C>T	p.Gln133X	Premature stop codon	7	1.44%
c.1951C>T	p.Arg651X	Premature stop codon	6	1.23%
c.2203C>T	p.Arg735X	Premature stop codon	6	1.23%
c.253_256delGAGT	p.Glu85MetfsX26	Deletion; frameshift	5	1.03%
c.1369_1372delCAAA	p.Gln457X	Premature stop codon	5	1.03%
c.1848C>A	p.Tyr616X	Premature stop codon	5	1.03%
c.987del	p.Ser329ArgfsX23	Deletion; frameshift	4	0.82%
c.1378+1G>C		Splice; donor	4	0.82%
c.2489+4A>C		Splice; donor	4	0.82%
c.2554delG	p.Glu852AsnfsX79	Premature stop codon	4	0.82%
	Del exon 2-14	CNV	3	0.62%
c.275T>A	p.Leu92X	Premature stop codon	3	0.62%
c.917_918delCC	p.Pro308GlnfsX29	Deletion; frameshift	3	0.62%
	Del exon 4	CNV	3	0.62%
c.1378G>A		Splice; donor	3	0.62%
c.1755_1756insTTGACTCA	p.Leu586ThrfsX72	Insertion; frameshift	3	0.62%
c.1821dupC	p.Val608Cysfs*6	Insertion; frameshift	3	0.62%
c.2013delC	p.Lys672ArgfsX12	Deletion; frameshift	3	0.62%
c.2176C>T	p.Gln726X	Premature stop codon	3	0.62%
c.218dupG	p.Asn74GlnfsX12	Insertion; frameshift	2	0.41%
c.224-3C>G		Splice; acceptor	2	0.41%

c.433_434delCT	p.Leu145GlufsX8	Deletion; frameshift	2	0.41%
c.663C>A	p.Tyr221X	Premature stop codon	2	0.41%
c.870G>A	p.Trp290X	Premature stop codon	2	0.41%
c.971_980del10	p.Ala324GlyfsX25	Deletion; frameshift	2	0.41%
c.1063C>T	p.Arg355X	Premature stop codon	2	0.41%
c.1170+1G>A		Splice; donor	2	0.41%
c.1171-2A>G		Splice; acceptor	2	0.41%
c.1630_1631insTT	p.Pro544LeufsX20	Insertion; frameshift	2	0.41%
c.1689-1G>C		Splice; acceptor	2	0.41%
c.1803delC	p.D601EfsX655	Deletion; frameshift	2	0.41%
c.1849C>T	p.Gln617*	Premature stop codon	2	0.41%
c.1978C>T	p.Gln660X	Premature stop codon	2	0.41%
c.2028G>A	p.Trp676X	Premature stop codon	2	0.41%
c.2034G>A	p.Trp678X	Premature stop codon	2	0.41%
c.2095C>T	p.Gln699X	Premature stop codon	2	0.41%
c.2119C>T	p.Gln707X	Premature stop codon	2	0.41%
c.2146-1_2146delGA		Splice; acceptor	2	0.41%
c.2216_2217delAT	p.His739ArgfsX3	Deletion; frameshift	2	0.41%
c.2421C>A	p.Tyr807X	Premature stop codon	2	0.41%
c.2426dupA	p.Asn809LysfsX18	Insertion; frameshift	2	0.41%
c.2490-1G>C		Splice; acceptor	2	0.41%
	Del exon 1-7	CNV	1	0.21%
	Del exon 1-4	CNV	1	0.21%
	5' UTR dupl	CNV	1	0.21%
c.14delG	p.Gly5fs	Deletion; frameshift	1	0.21%
c.175C>T	p.Gln59X	Premature stop codon	1	0.21%
c.184_185delCA	p.Gln62AspfsX23	Deletion; frameshift	1	0.21%
c.190dupC	p.Leu64ProfsX22	Insertion; frameshift	1	0.21%
c.219_223+5delCAACGGTGAG	p.Asn74GlyfsX	Deletion; frameshift (spl)	1	0.21%
c.223+1G>A		Splice; donor	1	0.21%
c.258T>G	p.Tyr86X	Premature stop codon	1	0.21%
c.268_272delACAC and 268_284Ins	p.His91fsx94	Ins/del; frameshift	1	0.21%
c.336+1G>A		Splice; donor	1	0.21%
c.337-2A>T		Splice; acceptor	1	0.21%
c.368G>A	p.Trp123X	Premature stop codon	1	0.21%
c.451delT	p.Ser151fs	Deletion; frameshift	1	0.21%
c.456delT	p.Asp153ThrfsX37	Deletion; frameshift	1	0.21%
c.498C>G	p.Tyr166X	Premature stop codon	1	0.21%
c.499T>A	p.Gln167X	Premature stop codon	1	0.21%
C.504C>G	p.Tyr168X	Premature stop codon	1	0.21%
c.508C>T	p.Gln170X	Premature stop codon	1	0.21%

c.517C>T	p.Gln173X	Premature stop codon	1	0.21%
c.534_535insCT	p.His179LeufsX12	Insertion; frameshift	1	0.21%
c.568delG	p.Val190CysfsX73	Deletion; frameshift	1	0.21%
c.587dup	p.Ser197PhefsX19	Insertion; frameshift	1	0.21%
c.604insG	p.Val202ValfsX13	Insertion; frameshift	1	0.21%
c.631C>T	p.Gln211X	Premature stop codon	1	0.21%
c.658C>T	p.Gln220X	Premature stop codon	1	0.21%
c.729C>G	p.Tyr243X	Premature stop codon	1	0.21%
c.771delG?	p.Lys258ArgfsX5?	Deletion; frameshift	1	0.21%
c.775G>T	p.Glu259X	Premature stop codon	1	0.21%
c.795_811del	p.Leu266Glnfsx64	Deletion; frameshift	1	0.21%
c.801delT	p.Thr268SerfsX34	Deletion; frameshift	1	0.21%
c.841delA	p.Thr281fs	Deletion; frameshift	1	0.21%
c.898_901delACGC	p.Thr300X	Premature stop codon	1	0.21%
c.951delG	p.His318Thrfsx2	Deletion; frameshift	1	0.21%
Not reported	p.His318TrpfsX10	Ins/del; frameshift	1	0.21%
c.968_971delAGGC	p.Gln323Argfs*28	Deletion; frameshift	1	0.21%
c.971_972insT	p.Ala324GlyfsX11	Insertion; frameshift	1	0.21%
c.982_983dupGG	p.Ser329GlufsX24	Insertion; frameshift	1	0.21%
c.1034+1G>T		Splice; donor	1	0.21%
c.1035-2A>T		Splice; acceptor	1	0.21%
c.1035-1G>A		Splice; acceptor	1	0.21%
c.1125-32del	p.Phe376AlafsX8	Deletion; frameshift	1	0.21%
c.1138G>T	p.Glu380X	Premature stop codon	1	0.21%
c.1170+1G>C		Splice; donor	1	0.21%
c.1177C>T	p.Gln393X	Premature stop codon	1	0.21%
c.1202_1209delTGCAGCTC	p.Leu401ProfsX6	Deletion; frameshift	1	0.21%
c.1205del	p.Gln402ArgfsX3	Deletion; frameshift	1	0.21%
c.1231dup	p.Val411GlyfsX15	Insertion; frameshift	1	0.21%
c.1234C>T	p.Gln412X	Premature stop codon	1	0.21%
c.1307_1315delins8	p.Leu436HisfsX11	Ins/del; frameshift	1	0.21%
c.1368delA	p.Lys456AsnfsX3	Deletion; frameshift	1	0.21%
c.1368insA	p.Gln456Thrfsx12	Insertion; frameshift	1	0.21%
c.1375delA	p.Thr459GlnfsX7	Deletion; frameshift	1	0.21%
	Del exons 6-11	CNV	1	0.21%
c.1511-2A>G		Splice; acceptor	1	0.21%
c.1597delA	p.Ile533SerfsX30	Deletion; frameshift	1	0.21%
c.1597_1600delATCC	p.Ile533ProfsX29	Deletion; frameshift	1	0.21%
c.1648_1657del	p.Leu550ThrfsX10	Deletion; frameshift	1	0.21%
c.1664delT	p.Phe555SerfsX8	Deletion; frameshift	1	0.21%
c.1688+1G>A		Splice; donor	1	0.21%
	Del exon 8-14	CNV	1	0.21%

c.1704delT	p.Gly569AlafsX8	Deletion; frameshift	1	0.21%
c.1709delC	p.Ala570ValfsX7	Deletion; frameshift	1	0.21%
c.1759delG	p.Val587SerfsX655	Deletion; frameshift	1	0.21%
c.1760delT	p.Val587Alafs*69	Deletion; frameshift	1	0.21%
c.1799delA	p.Asp600ValfsX56	Deletion; frameshift	1	0.21%
	Del exons 9-11	CNV	1	0.21%
c.1893T>G	p.Tyr631X	Premature stop codon	1	0.21%
c.1948delA	p.Ser650ValfsX6	Deletion; frameshift	1	0.21%
c.1969G>T	p.Glu657X	Premature stop codon	1	0.21%
	Del exon 10	CNV	1	0.21%
c.2009delA	p.Asn670ThrfsX14	Deletion; frameshift	1	0.21%
c.2058T>G	p.Tyr686X	Premature stop codon	1	0.21%
c.2076_2077delAA	p.Ser693CysfsX49	Deletion; frameshift	1	0.21%
c.2087dupA	p.Tyr697IlefsX46	Insertion; frameshift	1	0.21%
c.2145+1G>C		Splice; donor	1	0.21%
c.2145+3G>T		Splice; donor	1	0.21%
c.2179A>T	p.Lys727X	Premature stop codon	1	0.21%
c.2196_2197ins28	p.His732AlafsX9	Insertion; frameshift	1	0.21%
c.2918_2202delACACC	p.His733AlafsX8	Deletion; frameshift	1	0.21%
c.2240delA	p.Lys747ArgfsX7	Deletion; frameshift	1	0.21%
c.2299+1G>A		Splice; donor	1	0.21%
c.2447_2448delCC	p.Thr816ArgfsX10	Deletion; frameshift	1	0.21%
c.2489+1G>T		Splice; donor	1	0.21%
c.2544G>A	p.Trp848X	Premature stop codon	1	0.21%
c.2569_3018del50	p.Tyr857ArgfsX2	Deletion; frameshift	1	0.21%
c.2576delA	p.Lys859ArgfsX881+48	Deletion; frameshift	1	0.21%