Homozygous truncating variant in *PKP2* causes hypoplastic left heart syndrome

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Abstract

Left-sided congenital heart disease (LS-CHD) constitutes 14-20% of all congenital heart defects, and include bicuspid aortic valve, aortic valve stenosis, coarctation of the aorta, and hypoplastic left heart syndrome (HLHS). Isolated LS-CHD appears to have a complex origin. Nevertheless, familial clustering of left-sided heart defects strongly supports a genetic contribution. Severe outflow obstruction, in particular at the level of the aortic valve, is thought to lead to poor growth of the left ventricle in HLHS. However, HLHS may also result from a primary defect in myocardial development.

Heterozygous variants in the *PKP2* gene, encoding the desmosomal protein plakopholin-2, are associated with arrhythmogenic right ventricular cardiomyopathy. In contrast, evidence from animal studies suggest that loss of plakophilin-2 results in lethal defects in heart morphogenesis. We describe two siblings with HLHS and features of noncompaction due to a homozygous truncating variant in *PKP2*. Immunohistochemical analysis showed reduced expression of several desmosomal proteins as well as other intercalated disc components, including the major gap junction protein connexin-43. Our findings support previous observations that (i) plakophillin-2 is essential for cardiac morphogenesis, and (ii) abnormal myocardial development due to loss of functional plakophilin-2 may be the primary defect underlying some cases of HLHS.

Introduction

Hypoplastic left heart syndrome (HLHS) refers to a spectrum of cardiac malformations characterized by underdevelopment of the left heart structures, including varying degrees of hypoplasia of the left ventricle (LV), atresia, stenosis or hypoplasia of the aortic and/or mitral valve, and hypoplasia of the ascending aorta and aortic arch [1]. This is often accompanied by secondary endocardial fibroelastosis and right ventricular (RV) abnormalities [2, 3]. HLHS occurs in approximately 2-3 in 10,000 live births, accounting for 2-3% of all congenital heart disease [4]. Surgical treatment, either staged palliation or cardiac transplantation, has significantly improved survival: approximately half of the HLHS patients undergoing surgery will reach adult age [5].

Although the exact mechanisms underlying HLHS remain unclear, various theories have been proposed. It is suggested that LV hypoplasia is a secondary response to abnormal LV outflow tract development, resulting in altered flow dynamics and shear stress, and compromised ventricular growth and development [6]. In addition, LV hypoplasia may result from a primary defect in myocardial development. Like other left-sided heart defects, isolated HLHS appears to be highly heritable with evidence for autosomal dominant, autosomal recessive and polygenic inheritance [7-10]. However, only a few genes have been associated with isolated HLHS (*NOTCH1*, *NKX2-5*, *MYH6*, *HAND1*, *GJA1*, *SAP130* and *PCDHA13*), and only a small proportion of patients carry a pathogenic variant in one of these genes (**Table 1**) [11-18], suggesting further locus heterogeneity. We report, for the first time, a homozygous *PKP2* variant in two siblings with HLHS.

Table 1. Genes previously associated with isolated hypoplastic left heart syndrome

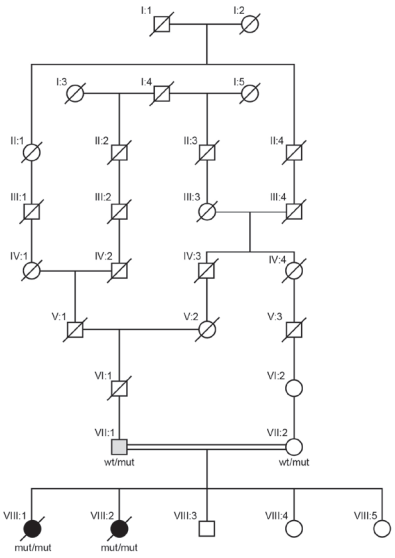
Gene	Location	Function	Genetic model	Reference
GJA1	6q22.31	Gap junction protein	Somatic	[17]
HAND1	5q33.2	Transcription factor	Somatic	[16]
NKX2-5	5q35.1	Transcription factor	Dominant	[13, 14]
NOTCH1	9q34.3	Transcription factor	Dominant, Recessive	[11, 12]
MYH6	14q11.2	Sarcomeric protein	Recessive	[15]
PCDHA13	5q31.3	Cell-adhesion protein	Digenic	[18]
SAP130	2q14.3	Splicing factor	Digenic	[18]

Methods

Clinical description

The first child of consanguineous Dutch parents was diagnosed with LV hypoplasia and hydrops fetalis at 35 weeks' gestation, resulting in intrauterine death three weeks later (**Figure 1A**). Autopsy showed atrial situs solitus with atrioventricular (AV) and ventriculo-arterial

(VA) concordance, enlargement of the right atrium, severe RV hypertrophy with prominent trabeculation, underdevelopment of the left heart with hypoplasia of the mitral valve, left ventricle, ostium aortae, ascending aorta and distal aortic arch, and multiple muscular ("swiss cheese") ventricular septal defects. No extracardiac malformations were seen. Cardiological examination of the parents, including transthoracic echocardiography, showed no abnormalities. In the second pregnancy, advanced ultrasound examination at 20 weeks of pregnancy did not reveal any abnormalities. However, repeat examination at 30 weeks' gestation again showed hypoplasia of the left ventricle and hydrops fetalis (**Figure 1B**). A female was born by cesarean section at 34 weeks. Her birth weight was 3530 gram (>98th percentile). Apgar scores were 3, 8 and 8 after 1, 5 and 10 minutes, respectively. She was intubated and admitted to the intensive care unit. Transthoracic echocardiography showed an abnormal myocardium and reduced contractility of both ventricles. Despite optimal medical therapy, cardiac function remained poor in the next weeks. The neonate died soon after withdrawal of treatment on day 19. Cardiac autopsy showed atrial situs solitus with AV and VA concordance, enlargement of the right atrium, dysplasia of the tricuspid valve leaflets, abnormal myocardium with prominent trabeculation of both ventricles, hypoplasia of the mitral valve, left ventricle, ascending aorta and aortic arch, mild subaortic stenosis, and a ventricular septal defect. Both pregnancies were complicated by severe maternal edema and pre-eclampsia (known as mirror or Ballantyne syndrome) [19]. Hereafter, the parents had three healthy children. In 2017, 18 years after the birth of their affected children, the parents re-contacted our department to get informed about new possibilities in genetic testing.



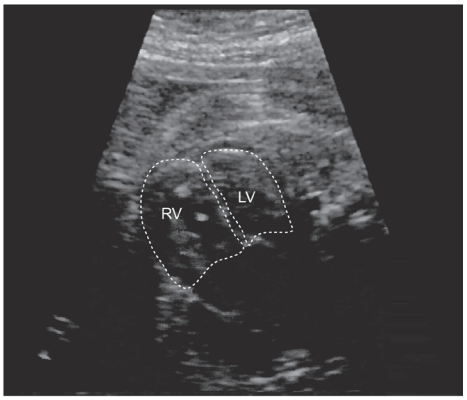


Figure 1. (A) Pedigree of the family with *PKP2*-related heart disease. Solid symbols indicate left ventricular hypoplasia. Grey symbol indicates arrhythmogenic right ventricular cardiomyopathy. (B) Prenatal ultrasound in individual VIII:2 showing prominent trabeculation and ventricular discrepancy with small left ventricle (LV) and enlarged right ventricle (RV).

Molecular studies

Next generation sequencing (NGS) of a panel of 52 cardiomyopathy-related genes was performed using targeted enrichment and paired-end sequencing on an Illumina MiSeq system (**Supplemental Table 1**). Because of the limited amount of DNA from both deceased children, analysis was started in DNA from peripheral blood samples from both parents. Sanger sequencing was performed to confirm NGS results and familial segregation. Disease-causing variants have been submitted to the respective Locus Specific Database (http://www.arcvdatabase.info). Written informed consent was obtained from both parents prior to their inclusion this study.

Histology

After gross macroscopic inspection, the heart specimens from the affected siblings (VIII:1 and VIII:2) were prepared for histologic examination. Paraffin-embedded tissue sections were stained with hematoxylin and eosin (H&E) and elastica-van Gieson (EvG) using standard techniques. Glutaraldehyde-fixed tissue samples were examined using electron microscopy.

Immunohistochemistry

Immunohistochemical analysis was performed on myocardial samples from individual VIII:2 and age-matched controls without overt heart disease, as described previously [20]. In brief, formalin-fixed, paraffin-embedded sections were deparaffinized and rehydrated before antibody retrieval. Primary antibodies included mouse monoclonal anti-plakoglobin (1:1,000 dilution), rabbit polyclonal anti-plakophilin 2 (1:50 dilution), mouse monoclonal anti-desmoplakin (1:10 dilution), mouse monoclonal anti-pan cadherin (1:400 dilution), rabbit polyclonal anti-43 (1:400 dilution), and rabbit polyclonal anti-SAP97 (1:100 dilution). To be able to identify the truncated plakophilin-2 protein (if expressed), we selected an antibody directed against an epitope in the N-terminal region (NBP1-86078, Novus Biologicals). The slides were then incubated with indocarbocyanine-conjugated goat anti-mouse or goat anti-rabbit secondary antibodies (1:400 dilution). Immunostained preparations were analyzed by confocal microscopy.

Literature review

We searched PubMed and Google Scholar to identify relevant articles reporting the clinical and genetic characteristics of patients with biallelic *PKP2* variants. Search terms included: “PKP2”, “plakophilin-2”, “recessive”, “homozygous”, “compound heterozygous” and “biallelic”. Searches were limited to English language articles published up to December 2017. We also assessed the references cited by these articles.

Results

Genetic analysis

Both parents were found to carry a heterozygous variant c.1211dup in the *PKP2* gene (NM_004572.3) encoding the desmosomal protein plakophilin-2. This variant was present in homozygous state in their two affected daughters. The duplication produces a frameshift that is predicted to result in a premature termination codon four amino acids downstream p.(Val406fs), within the second armadillo domain. The abnormal transcript is expected to undergo nonsense-mediated mRNA decay. The same variant has been reported previously in heterozygous state in 14 index patients with arrhythmogenic right ventricular cardiomyopathy (ARVC) [21-23]. Six of these patients carried an additional potentially pathogenic variant in *DSC2* or *DSP* (**Supplemental Table 2**). The variant was present twice in our in-house database encompassing 975 cardiomyopathy patients, and absent from population databases. According to the guidelines of the American College of Medical Genetics and Genomics [24], this variant was classified as "pathogenic" (class 5).

Cardiac examination

Cardiac re-examination in the father showed mild dilatation of the RV (end-diastolic volume to BSA 108 ml/m^2) with regional dyskinesia of the RV outflow tract, and late potentials by signal-averaged ECG (3/3 parameters), therefore fulfilling the modified Task Force criteria for a definite diagnosis of ARVC [25]. Cardiac re-examination in the mother showed regional RV dyskinesia without dilatation or reduced ejection fraction, and late potentials by signal-averaged ECG (3/3 parameters), classified as borderline ARVC. The healthy siblings (aged 6, 9 and 10 years) did not show any signs of cardiac disease. Therefore, the parents decided to postpone genetic testing in these children. Subsequent family screening revealed variable features of ARVC in other heterozygous relatives (data not shown).

Histopathology

Re-examination of both heart specimens showed LV hypoplasia and biventricular noncompaction (**Figure 2**). Microscopic examination confirmed the presence of prominent trabeculae, deep intertrabecular recesses, and extensive endocardial fibroelastosis (**Figure 3A** and **3B**). We found no evidence of myocyte disarray or fatty infiltration of the myocardium. The aortic arch showed normal wall morphology (data not shown), suggesting that the hypoplasia is the effect of reduced blood flow rather than an intrinsic defect. Electron microscopic examination of cardiac tissue samples from individual VIII:2 revealed disorganization of the sarcomeric structure and tightly packed mitochondria of varying sizes with abnormal cristae structure and focal condensations (**Figure 3C and 3D**). Intercalated discs were markedly reduced in number and often had an irregular and fragmented appearance.

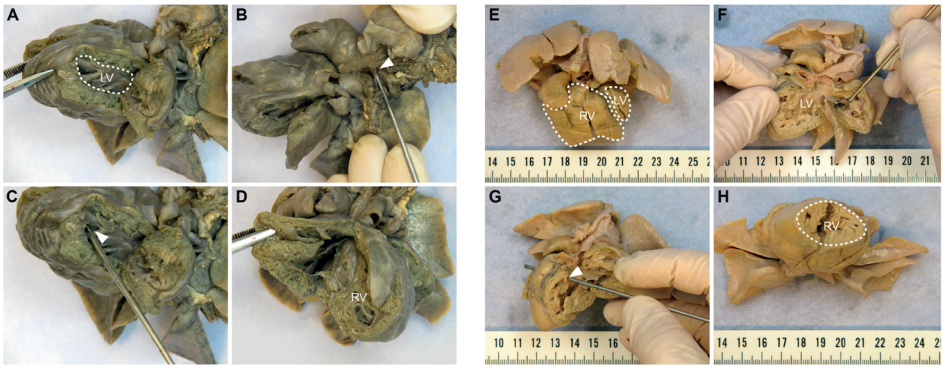


Figure 2. Macroscopic examination of the heart from individual VIII:1 showing hypoplasia of the (A) left ventricle and (B) aortic arch, (C) ventricular septal defects, and (D) right ventricular hypertrophy. Macroscopic examination of the heart from individual VIII:2 showing (E) ventricular discrepancy (apex removed), (F) hypoplastic left ventricle with prominent trabeculation, (G) ventricular septal defect, and (H) right ventricular hypertrophy.

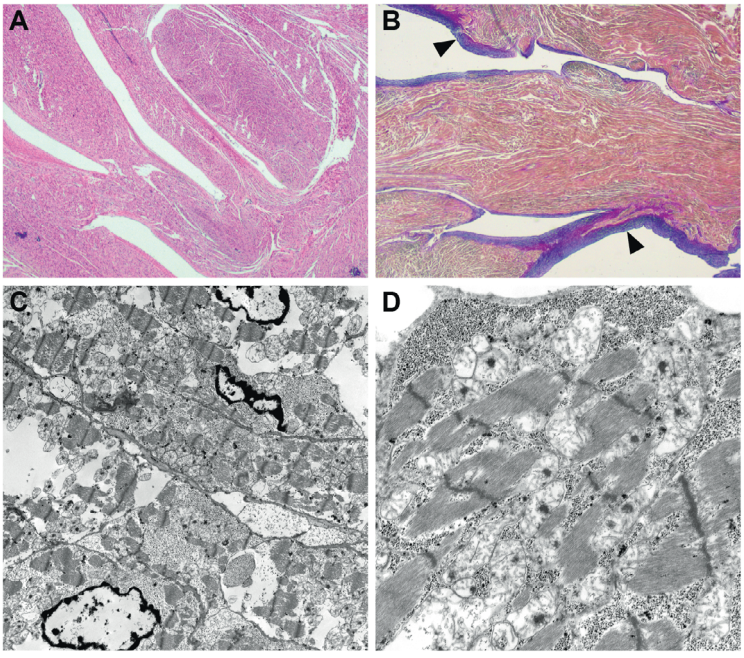


Figure 3. Microscopic examination of the myocardium. (A-B) Histology of noncompaction (individual VIII:2, H&E stain), with endocardial fibroelastosis (individual VIII:1, EvG stain). (C-D) Electron microscopic examination (magnification x 4,400 and 11,000, respectively) in individual VIII:2 showing sarcomeric disorganization and severely abnormal mitochondria. Intercalated discs were barely recognizable.

Immunohistochemical analysis

To determine the effect of loss of plakophilin-2 on the expression of other junctional proteins, we performed immunohistochemical analysis of cardiac tissue samples of left and right ventricular mycoardium from both affected siblings and age-matched controls. Experiments in-

volving samples from individual VIII:1 failed on repeated attempts, probably due to low tissue quality. As illustrated in **Figure 4**, samples from individual VIII:2 showed strong signal levels for the non-desmosomal adhesion molecule N-cadherin, which was indistinguishable from that in control samples. By contrast, immunoreactive signals for other junctional proteins, including plakoglobin, desmoplakin, plakophilin-2, connexin-43 and synapse-associated protein 97 (SAP97), were absent or greatly reduced when compared to those in control samples.

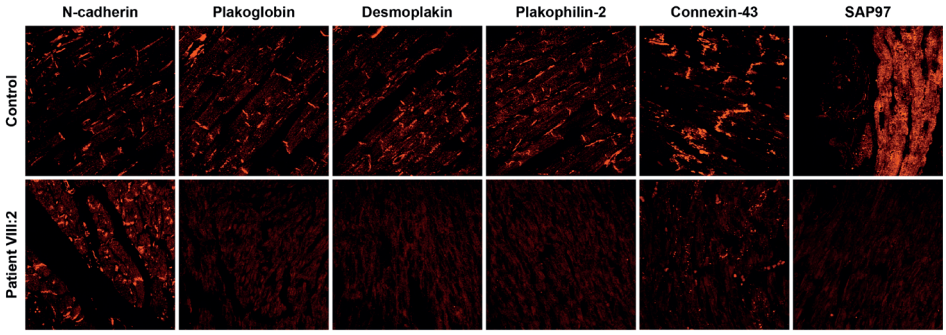


Figure 4. Immunohistochemical analysis of intercalated disc proteins in individual VIII:2. Confocal immunofluorescence images showing immunoreactive signal levels for N-cadherin at the intercalated discs in the patient are similar to those in the control sample, whereas signal levels for the junctional proteins plakoglobin, desmoplakin, plakophilin-2, connexin-43 and SAP97 are either absent or clearly reduced in comparison to those in the control sample.

Genotype-phenotype correlation

We collected 30 cases with (likely) pathogenic homozygous or compound heterozygous *PKP2* variants from international literature (**Table 2**). In general, individuals carrying double variants displayed a severe ARVC phenotype. The majority of these individuals carried at least one missense variant. Two young individuals presented with acute myocarditis [26]. Intriguingly, Ramond et al. recently reported two siblings with severe LV noncompaction due to a homozygous *PKP2* deletion [27].

Table 2. Clinical and genetic characteristics of index patients with biallelic *PKP2* variants[a]

***PKP2* variant 1**	***PKP2* variant 2**	**Additional variant(s)**	**Phenotype**	**Age at dx**	**Reference**
Double truncating mutations or deletions					
c.1211dup p.(Val406fs)	*c.1211dup p.(Val406fs)*	N/A	HLHS, LVNC	neonatal	This study
whole gene deletion	whole gene deletion	N/A	LVNC	neonatal	[27]
c.508C>T p.(Gln170)*	*c.508C>T p.(Gln170*)*	*JUP* c.2078A>G p.(Tyr693Cys)	ARVC	unknown	[36]
c.2484C>T abnormal splice product[c]	*c.2484C>T abnormal splice product*[c]	N/A	ARVC	44 years	[34, 54-56]
c.2577+1G>T abnormal splice product	*c.2577+1G>T abnormal splice product*	N/A	ARVC	32 years	[35]

Table 2. Clinical and genetic characteristics of index patients with biallelic *PKP2* variants[a] (*continued*)

***PKP2* variant 1**	***PKP2* variant 2**	**Additional variant(s)**	**Phenotype**	**Age at dx**	**Reference**
Other compound heterozygous or homozygous mutations					
c.145_148del p.(Thr50fs)	c.1592T>G p.(Ile531Ser)[b]	N/A	ARVC	32 years	[31]
c.184C>A p.(Gln62Lys)	c.1839C>G p.(Asn613Lys)	N/A	ARVC	32 years	[57]
c.184C>A p.(Gln62Lys)	*c.2119C>T p.(Gln707*)*[b]	N/A	ARVC	36 years	[33]
c.184C>A p.(Gln62Lys)	*c.2119C>T p.(Gln707*)*	*DSP* c.4961T>C p.(Leu1654Pro)	ARVC	31 years	[31, 33, 58]
c.397C>T p.(Gln133)*	c.2615C>T p.(Thr872Ile)[b]	N/A	ARVC	unknown	[55, 56]
c.397C>T p.(Gln133)*	c.2615C>T p.(Thr872Ile)[b]	N/A	ARVC	unknown	[55, 56]
c.397C>T p.(Gln133)*	c.2615C>T p.(Thr872Ile)[b]	N/A	ARVC	unknown	[56]
c.397C>T p.(Gln133)*	c.2615C>T p.(Thr872Ile)[b]	*DSG2* c.1480G>A p.(Asp494Asn)	ARVC	unknown	[55, 56]
c.419C>T p.(Ser140Phe)	*c.2146-1G>C abnormal splice product*	*DSG2* c.166G>A p.(Val56Met)	ARVC	50 years	[31, 59]
c.427C>T p.His143Tyr	*c.2554del p.Glu852fs*	N/A	ARVC	unknown	[36]
c.627C>G p.(Ser209Arg)	*c.2447_2448del p.(Thr816fs)*	*DSG2* c.437G>A p.(Arg146His)	ARVC	44 years	[31, 33]
c.631C>T p.(Gln211)*	c.2333T>C p.(Ile778Thr)[b]	*DSP* c.3764G>A p.(Arg1255Lys)	ARVC	40 years	[31, 33]
c.746G>A p.(Ser249Asn)	Deletion exon 8[b]	N/A	ARVC	unknown	[55, 56]
c.976G>A p.(Ala326Thr)	c.976G>A p.(Ala326Thr)	*DSP* c.593A>C p.(Gln189Pro)[d]	ARVC	unknown	[60]
c.1114G>C p.(Ala372Pro)	*c.2146-1G>C abnormal splice product*[b]	*DSP* c.4609C>T p.(Arg1537Cys)	ARVC	41 years	[31]
c.1162C>T p.(Arg388Trp)	*c.2197_2202delinsG p.(Ala733fs)*	N/A	ARVC	childhood	[61]
c.1162C>T p.(Arg388Trp)	*c.2509del p.(Ser837fs)*	N/A	ARVC	16 years	[31]
c.1592T>G p.(Ile531Ser)	c.2359C>T p.(Leu787Phe)[b]	N/A	ARVC	52 years	[31]
c.1613G>A p.(Trp538)*	c.1914G>C p.(Gln638His)[b]	*DSP* c.88G>A p.(Val30Met)	ARVC	30 years	[31]
c.1694T>G p.(Met565Arg)	c.2434G>A p.(Asp812Asn)	N/A	ARVC	76 years	[32]
c.1749T>G p.(Ile583Met)	*c.2489+1G>A abnormal splice product*	N/A	ARVC	unknown	[36]
c.2035C>T p.(His679Tyr)	c.2035C>T p.(His679Tyr)	*LAMA4* c.133C>T p.(Gln45*)	DCM	unknown	[62]
c.2062T>C p.(Ser688Pro)	c.2485G>A p.(Asp829Asn)	N/A	AM	16 years	[26]
c.2434G>A p.(Asp812Asn)	*c.2489+1G>A abnormal splice product*	N/A	ARVC	20 years	[32]
c.2519C>T p.(Ala840Val)	c.2519C>T p.(Ala840Val)	N/A	DCM/AM	11 years	[26]

AM, acute myocarditis; ARVC, arrhythmogenic right ventricular cardiomyopathy; DCM, dilated cardiomyopathy; dx, diagnosis; HLHS, hypoplastic left heart syndrome; LNVC, left ventricular noncompaction; N/A, not ascertained or not applicable. Reference sequences: NM_001943.3 (*DSG2*), NM_004415.2 (*DSP*), NM_021991.2 (*JUP*), NM_001105206.1 (*LAMA4*), NC_000012.12, NM_004572.3 (*PKP2*). Truncating mutations expected to result in functional null alleles are displayed in italic. [a] The pathogenicity of some of the missense variants listed here is uncertain. [b] Not clear whether these variants are in *cis* or in *trans*. [c] Translationally silent nucleotide change that creates a cryptic splice site, resulting in a 7 base pair deletion in approximately 80% of the transcripts. [d] This variant was present in homozygous state.

Discussion

We describe two siblings with HLHS due to a homozygous truncating variant in the *PKP2* gene. Both individuals displayed diffuse myocardial abnormalities with features of noncompaction, and died in the neonatal period. Plakophilin-2 is the primary cardiac plakophilin, an essential component of desmosomes. Heterozygous variants in the corresponding gene (*PKP2*) have been found in patients with arrhythmogenic right ventricular cardiomyopathy (ARVC, MIM 609040), a genetically heterogeneous disorder characterized by progressive fibrofatty replacement of the myocardium that predisposes to ventricular tachyarrhythmias and sudden cardiac death [28]. As of December 2017, over 283 different *PKP2* variants have been included in the ARVD/C Genetic Variants Database [29]. Of these, 171 variants have been classified as pathogenic; the significance of the other variants is still unknown. Pathogenic variants, the majority of which are nonsense, frameshift or splice site variants that result in a truncated protein, are scattered along the entire coding region of the *PKP2* gene. In general, heterozygous pathogenic variants are not fully penetrant and show considerable phenotypic intrafamilial variability. Nonsense variants in *PKP2* have been associated with later onset of ARVC [30]. Biallelic or digenic variants in desmosomal genes may result in a more severe disease phenotype [31-33].

We identified 29 additional cases with biallelic variants in *PKP2* in the literature (**Table 2**). Four of these cases carried a double truncating variant or deletion expected to result in complete absence or severe reduction of protein expression. Ramond et al. recently reported a homozygous *PKP2* gene deletion in two siblings with lethal noncompaction cardiomyopathy [27], showing considerable phenotypic overlap with the family described in this study. The other three cases, however, displayed an ARVC phenotype. Two cases carried a homozygous variant that causes aberrant splicing of the *PKP2* transcript. These variants might produce a small proportion of normally spliced transcripts, leaving sufficient residual protein activity for normal myocardial development but resulting in ARVC later in life. Indeed, Awad et al. demonstrated that approximately 20% of transcripts derived from the c.2484C>T variant allele are transcribed normally [34]. No gene expression studies were performed for the c.2577+1G>T variant [35]. The c.508C>T variant, on the other hand, results in a premature termination codon [36]. The product will likely undergo rapid degradation by nonsense-mediated mRNA decay (NMD). However, albeit rare, mechanisms such as exon skipping or alternative translation at a downstream start site might result in the production of truncated protein and partial rescue of the phenotype, i.e. resulting in adult-onset ARVC instead of a lethal cardiac malformation [37, 38].

Desmosomes are essential during embryonic development, providing both tissue integrity and flexibility required for proper morphogenesis and patterning, and acting as cell signaling

regulators [39, 40]. Studies using animal models have already shown that plakophilin-2 is essential for cardiac morphogenesis. Plakophilin-2 deficient mice showed reduced thickening of the atrial walls and reduced trabeculation of the ventricles, followed by blood leakage into the pericardial and peritoneal cavities and, subsequently, embryonic lethality at mid-gestation (mouse embryonic day 10.5 to 12) [41]. In the mutants, desmoplakin was dissociated from the junctional proteins but instead appeared as granular aggregates in the cytoplasm. Cytoskeletal architecture was clearly disturbed. Knockdown of plakophilin-2 in zebrafish resulted in severe cardiac defects, including cardiac edema, atrial enlargement and looping defects [42]. The cardiac valves appeared normal. Morphant hearts had fewer desmosomes, reduced intercellular junction elements and larger gaps between the cells. Adherens junctions were unaffected.

Immunohistochemical analysis in one of our patients showed that, besides plakophilin-2, other nonmutant desmosmal proteins failed to localize at the intercellular adhesion junctions. In addition, signals for the major gap junction protein connexin-43 (Cx43) and the PDZ-containing protein SAP97 were also reduced at the intercalated discs. These findings are in line with previous observations that variants in a single desmosomal gene cause subcellular redistribution of other desmosomal proteins and SAP97, as well as diffuse remodeling of gap junctions [20, 43]. Gap junction remodeling is an early manifestation in ARVC. However, little is known about the exact underlying mechanisms by which mutant desmosomal proteins result in gap junction remodeling. Numerous studies have indicated that intercellular mechanical coupling is essential for normal electrical coupling (summarized by [44]). PKP2 silencing leads to Cx43 remodeling, even if cardiac cells are not subjected to the mechanical forces generated by the beating heart [45, 46]. The reduced expression of connexin-43 seems to be the result of altered gene transcription [47]. Additional studies have shown that PKP2 and Cx43 co-exist in the same macromolecular complex, suggesting that connexin-43 remodeling might also be a direct effect of PKP2 disruption [45, 48, 49].

Reduced expression of connexin-43 is not specific for ARVC, but is also observed in a variety of other heart diseases, including hypertrophic cardiomyopathy, ischemic heart disease and myocarditis [50]. Disturbed connexin-43 expression had also been reported in severely hypoplastic left hearts [51]. Somatic variants in the *GJA1* gene, encoding connexin-43, have been identified in heart tissue from patients with HLHS [17]. *Gja1* knockout mice show abnormal outflow tract development [52]. These observations suggest that HLHS in our family might also (partially) result from decreased connexin-43 expression.

In conclusion, our study demonstrates that biallelic truncating variants in *PKP2* are associated with HLHS with features of noncompaction. Our findings support previous observations that (i) plakophillin-2 is essential for cardiac morphogenesis, and (ii) abnormal myocardial develop-

ment due to loss of functional plakophilin-2 may be the primary defect underlying HLHS [53]. This genetic form of HLHS might be recognized by the coexistence of noncompaction of the myocardium, and could be overrepresented in perinatal deaths. The exact mechanisms resulting in abnormal myocardial formation and subsequent HLHS remains to be determined.

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Supplemental Information

Supplemental Table 1. Cardiomyopathy panel version 5 (52 genes)

Gene	Protein	Reference
ABCC9	ATP binding cassette subfamily C member 9	NM_020297.2
ACTC1	actin, alpha, cardiac muscle 1	NM_005159.4
ACTN2	actinin alpha 2	NM_001103.2
ALPK3	alpha kinase 3	NM_020778.4
ANKRD1	ankyrin repeat domain 1	NM_014391.2
BAG3	BCL2 associated athanogene 3	NM_004281.3
CALR3	calreticulin 3	NM_145046.3
CAV3	caveolin 3	NM_033337.2
CRYAB	crystallin alpha B	NM_001885.1
CSRP3	cysteine and glycine rich protein 3	NM_003476.3
CTNNA3	catenin alpha 3	NM_013266.2
DES	desmin	NM_001927.3
DSC2	desmocollin 2	NM_024422.3
DSG2	desmoglein 2	NM_001943.3
DSP	desmoplakin	NM_004415.2
EMD	emerin	NM_000117.2
FHL1	four and a half LIM domains 1	NM_001159702.2
GLA	galactosidase alpha	NM_000169.2
JPH2	junctophilin 2	NM_020433.4
JUP	junction plakoglobin	NM_021991.2
LAMA4	laminin subunit alpha 4	NM_001105206.1
LAMP2	lysosomal associated membrane protein 2	NM_002294.2
LDB3	LIM domain binding 3	NM_007078.2
LMNA	lamin A/C	NM_170707.2
MIB1	mindbomb E3 ubiquitin protein ligase 1	NM_020774.2
MYBPC3	myosin binding protein C, cardiac	NM_000256.3
MYH6	myosin heavy chain 6	NM_002471.3
MYH7	myosin heavy chain 7	NM_000257.2
MYL2	myosin light chain 2	NM_000432.3
MYL3	myosin light chain 3	NM_000258.2
MYOZ2	myozenin 2	NM_016599.3
MYPN	myopalladin	NM_032578.2
NEXN	nexilin F-actin binding protein	NM_144573.3
NKX2-5	NK2 homeobox 5	NM_004387.3
PKP2	plakophilin 2	NM_004572.3
PLN	phospholamban	NM_002667.3
PRDM16	PR/SET domain 16	NM_22114.3
PRKAG2	protein kinase AMP-activated non-catalytic subunit gamma 2	NM_016203.3
RBM20	RNA binding motif protein 20	NM_001134363.1
RYR2	ryanodine receptor 2	NM_001035.2

Supplemental Table 1. Cardiomyopathy panel version 5 (52 genes) (*continued*)

Gene	Protein	Reference
SCN5A	sodium voltage-gated channel alpha subunit 5	NM_198056.2
TAZ	tafazzin	NM_000116.3
TBX20	T-box 20	NM_001077653.2
TCAP	titin-cap	NM_003673.3
TMEM43	transmembrane protein 43	NM_024334.2
TNNC1	troponin C1, slow skeletal and cardiac type	NM_003280.2
TNNI3	troponin I3, cardiac type	NM_000363.4
TNNT2	troponin T2, cardiac type	NM_001001430.1
TPM1	tropomyosin 1 (alpha)	NM_001018005.1
TTN	titin	NM_001267550.1
TTR	transthyretin	NM_000371.3
VCL	vinculin	NM_014000.2

Test description: regions of interest were enriched using a custom-designed SureSelect library (Agilent Technologies, Santa Clara, CA, USA) and subsequently sequenced on an MiSeq platform (Illumina, San Diego, CA, USA). The sequence reads were mapped against the human reference genome GRCh37/hg19 with the Burrows-Wheeler Aligner and analyzed using SeqPilot version 4.1.2 (JSI medical system GmbH, Ettenheim, Germany).

Supplemental Table 2. Previous reports of the *PKP2* c.1211 dup p.(Val406fs) variant

Patients	Origin	Additional variant(s)	Reference
n=1	United States	*DSP* c.269A>G	[1]
n=1	Italy	*DSC2* c.2686_2687dup	[2]
n=11	Netherlands	*DSC2* c.2194T>G *DSC2* c.2686_2687dup *DSP* c.269A>G *DSP* c.5218G>A	[3]
n=1	United States	None	[4]

Note: this variant has also been reported as c.1211_1212insT p.(V406SfsX3) and c.1212insT, p.(L404fsX409).

Supplemental References

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