Clinical Letter

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

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Hypoplastic left heart syndrome (HLHS) refers to a spectrum of cardiac malformations characterized by underdevelopment of the left heart structures. Like other left-sided heart defects, isolated HLHS appears to be highly heritable. However, only a few genes have been associated with isolated HLHS, and only a small proportion of patients carry a pathogenic variant in one of these genes, suggesting further locus heterogeneity. Here, we report two siblings with HLHS and features of noncompaction resulting from a homozygous truncating variant in the *PKP2* gene.

The first child of consanguineous Dutch parents (**Figure [A]**) was diagnosed with left ventricular hypoplasia and hydrops fetalis, resulting in intrauterine death at 38 weeks’ gestation. Autopsy revealed additional severe right ventricular hypertrophy with prominent trabeculation and multiple ventricular septal defects. No extracardiac malformations were reported. Cardiological examination of the parents (age 28 and 30 years) showed no abnormalities. In the second pregnancy, ultrasound examination again showed hypoplasia of the left ventricle and hydrops fetalis. Postnatal transthoracic echocardiography showed an abnormal myocardium with prominent trabeculation and reduced contractility of both ventricles. Because cardiac function did not improve despite optimal medical therapy, treatment was withdrawn, and the patient subsequently died at age 19 days. Cardiac autopsy confirmed the clinical findings (**Figure [B]**). Hereafter, the parents had three healthy children.

Considering the unusual aspect of the myocardium, we hypothesized that the HLHS resulted from an intrinsic defect in myocardial development. Targeted next-generation sequencing of cardiomyopathy-related genes revealed a heterozygous variant c.1211dup in the *PKP2* gene (NM\_004572.3), encoding the desmosomal protein plakophilin-2, in both parents. 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),1 and was classified as “pathogenic” according to 2015 ACMG guidelines. Upon cardiac re-examination at age 46 and 48 years, both parents indeed showed signs of ARVC.

Plakophilin-2 is the primary cardiac plakophilin, an essential component of desmosomes. Heterozygous variants in the corresponding *PKP2* gene have been found in patients with ARVC (MIM 609040), a genetically heterogeneous disorder characterized by progressive fibrofatty replacement of the myocardium that predisposes to ventricular tachyarrhythmias and sudden cardiac death. As of October 2018, over 283 different *PKP2* variants have been included in the ARVD/C Genetic Variants Database.1 Of these, 171 variants have been classified as pathogenic; the significance of the other variants is still unknown. Pathogenic variants are scattered along the entire coding region of the *PKP2* gene. In general, these variants – in heterozygous state – are not fully penetrant and show considerable phenotypic intrafamilial variability.

Using systematic literature review, we identified 29 additional cases with homozygous or compound heterozygous *PKP2* variants. In general, individuals carrying double variants displayed a severe ARVC phenotype. The majority of these individuals carried at least one missense variant. Ramond et al. recently reported two siblings with severe LV noncompaction due to a homozygous *PKP2* deletion.2 These observations suggest that the phenotype depends on the level of residual activity, wherein complete loss results in a lethal cardiac malformation. Indeed, studies in animal models confirm that plakophilin-2 is essential for cardiac morphogenesis.3, 4

We studied the effect of loss of plakophilin-2 on the cardiac intercalated discs in both affected siblings and age-matched controls. Electron microscopic examination revealed disorganization of the sarcomeric structure. Intercalated discs were reduced in number and often had an irregular and fragmented appearance (**Figure [C]**). Immunohistochemical analysis 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 and the PDZ-containing protein SAP97 were also reduced at the intercalated discs (**Figure [D]**). These findings confirm previous observations that variants in a single desmosomal gene cause subcellular redistribution of other desmosomal proteins, as well as diffuse remodeling of gap junctions.

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**CONFLICT OF INTEREST**

The authors declare no competing financial interests.

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**FIGURE LEGEND**

**Clinical and histopathologic characteristics. A**, Pedigree of the family. Squares and circles represent males and females, respectively. Solid symbols indicate left ventricular (LV) hypoplasia. Grey symbol indicates arrhythmogenic right ventricular (RV) cardiomyopathy. *PKP2* genotypes: mut, mutant allele; wt, wild-type allele. **B**, Macroscopic examination of the heart from patient VIII:2 showing hypoplasia of the LV, and RV hypertrophy. **C**, Microscopic examination of the myocardium. Elastica-van Gieson staining showing endocardial fibroelastosis (**upper panel**, arrow heads). Electron microscopy showing sarcomeric disorganization and abnormal intercalated discs (**lower panel**, x11,000 magnification). **D**, Confocal immunofluorescence images showing absent or clearly reduced signal levels for the junctional proteins plakoglobin, desmoplakin, plakophilin-2, connexin-43 and SAP97 in the patient compared with control sample.