CASE REPORT

Sudden Unexpected Death of Infantile Dilated Cardiomyopathy with JPH2 and PKD1 Gene Variants

Aya Miura,1* PhD, Hidehito Kondo,2* MD, Takuma Yamamoto,1 MD, Yasuko Okumura,2 MD and Hajime Nishio,1 MD

Summary
A Japanese girl with polycystic kidney disease (PKD) developed normally, but at 8 months of age, she was hospitalized for acute onset dyspnea. On the day after admission to hospital, her general condition suddenly became worse. An echocardiogram showed left ventricular dilatation with thin walls, severe mitral valve regurgitation, and a reduced ejection fraction. She died of acute cardiac failure 3 hours after the sudden change. Post-mortem analysis with light microscopy showed disarray of cardiomyocytes without obvious infiltration of lymphocytes, and we diagnosed her heart failure as idiopathic dilated cardiomyopathy (DCM). Clinical exome sequencing showed compound heterozygous variants in JPH2 (p.T237A/p.I414L) and a heterozygous nonsense mutation in PKD1 (p.Q4193*). To date, several variants in the JPH2 gene have been reported to be pathogenic for adult-onset hypertrophic cardiomyopathy or DCM in an autosomal dominant manner and infantile-onset DCM in an autosomal recessive manner. Additionally, autosomal dominant polycystic kidney disease is a systemic disease associated with several extrarenal manifestations, such as cardiomyopathy. Here we report a sudden infant death case of DCM and discuss the genetic variants of DCM and PKD.

Key words: Clinical exome sequence, Compound heterozygous variants, PKD-cardiomyopathy, Polycystic kidney disease, Postmortem genetic analysis

Dilated cardiomyopathy (DCM) is the most common cardiomyopathy in children and the majority of patients present before 1 year old.1) Junctophilin-2, which is encoded by JPH2, connects the L-type calcium channel in the cell membrane and the ryanodine receptor in the sarcoplasmic reticulum for calcium-induced calcium release (CICR).2,3) To date, several variants in the JPH2 gene have been reported to be pathogenic mutations for adult-onset hypertrophic cardiomyopathy or DCM in an autosomal dominant manner.4,5) Recently, an infantile-onset DCM case with a homozygous nonsense variant in the JPH2 gene was reported, which suggested that JPH2 variants, mainly associated with adult-onset dominant disorders, cause childhood-onset recessive DCM.6,7) Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disorder, and it is caused by mutations to the polycystic kidney disease 1 gene (PKD1) or PKD2.8) PKD1 encodes polycystin-1 (PC1), which is a transmembrane protein in the cell membrane and primary cilia. Polycystins (PCs) are expressed in many tissues, including tubular epithelia, as well as cardiomyocytes.9,10) ADPKD is a systemic disease associated with several extrarenal manifestations, such as cardiomyopathy.10,11) Recently, the concept of “polycystic kidney disease (PKD) cardiomyopathy” has been proposed12,13) and PKD is sometimes associated with DCM.

We report here an infantile case of sudden cardiac death in an infant who was diagnosed with DCM with JPH2 gene variants at postmortem examinations. She also had ADPKD with the PKD1 gene variant. To the best of our knowledge, this is the first case of DCM with JPH2 and PKD1 gene variants. We discuss the association between cardiomyopathy and these variants.

Case Report
A Japanese girl was born at term as the first child to non-consanguineous healthy parents. She developed neonatal jaundice and was treated with phototherapy at 4 days old. Ultrasound imaging for a screening examination on admission showed clusters of cysts in both kidneys. Although an obvious abnormality was not recognized in the heart at this time, a postmortem review of the echocardiogram showed slight noncompaction of the left ven-
tricular myocardium (Figure 1A). She was diagnosed with PKD and her renal function was normal. She developed normally and showed no clinical findings of cardiac dysfunction in infancy. At 8 months of age, she visited her family doctor due to wheezing, chest retractions, and chest protrusion. She was prescribed bronchodilators. The next evening, her respiratory condition became worse, and she was admitted to our hospital. Laboratory tests at admission showed an elevated white blood cell count (16,920 /μL) and a mild degree of acidosis (pH: 7.316, PvCO2: 34.3 mmHg, HCO3−: 16.9 mmol/L, base excess: −7.9 mmol/L). Creatine kinase and C-reactive protein levels were not increased. Chest radiographs showed cardiomegaly without lung congestion or consolidation (Figure 1B). An intravenous drip, administration of systemic glucocorticoids, and bronchodilator inhalation were initiated. The next morning, her general condition suddenly worsened, and her oxygen saturation had decreased to 70%. An echocardiogram showed left ventricular dilatation with thin walls, severe mitral valve regurgitation, and a reduced ejection fraction (30%) (Figure 1C). Cardiac function was not improved after dopamine injection. Immediately after initiation of artificial ventilation, cardiopulmonary rescue was started for severe bradycardia. She died of acute cardiac failure 3 hours after this sudden change.

**Postmortem clinical examination:** Needle biopsy of the left ventricular myocardium was performed with written informed consent. Light microscopy showed no findings of obvious infiltration of lymphocytes with myocardial injury, but cardiomyocyte disarray was observed (Figure 1D). Postmortem analysis of the patient’s serum at admission and the next day showed extremely elevated N-terminal pro-brain natriuretic peptide levels (14,400 pg/mL and > 35,000 pg/mL, respectively), but no elevation in creatine kinase and troponin-T levels. Neither bacteria nor a virus was detected from the patient’s sample. The acylcarnitine profile of the serum showed no specific abnormality. Mitochondrial analysis of cardiomyocytes excluded mitochondrial respiratory chain complex deficiency. These findings suggested that the contractile dysfunction was not related to either infection or metabolic disease. We diagnosed the heart failure as an idiopathic DCM, rather than acquired cardiomyopathy.

**Postmortem genetic analysis:** Recent studies have determined the genetic basis of cardiomyopathy not only in familial, but also sporadic, cases. Therefore, we performed clinical exome sequencing using the patient’s and parents’ blood to clarify the genetic cause of DCM, although there was no family history of DCM. Written informed consent was obtained from the parents. The use of patient-derived samples and genomic analysis were approved by the Ethics Committee of Hyogo College of Medicine (Permit Number: 370) and Red Cross Kyoto Daiichi Hospital (Permit Number: 778).

Clinical exome sequencing (TruSight One sequencing panel; Illumina, San Diego, CA, USA) of the patient and the parents was performed on the Illumina MiSeq (Illumina) as previously described. A list of genes of TruSight One is available online at http://products.illumina.com/products/trusight-one-sequencing-panel.html. On average, approximately 11 million total reads were produced and approximately 8 million reads mapped to the targeted
region in the sample. The mean coverage of the coding sequence was 66.5 reads, with an overall average gene level coverage at ≥20 reads of 86.7%. There were 6652 single-nucleotide variants, 87 insertions, and 87 deletions detected. Among these variants, synonymous variants without amino acid changes were excluded. After filtering steps as previously described, the total number of detected variants was 535, including 12 stop-gained variants and 358 missense variants.

Among cardiomyopathy-related genes, we identified compound heterozygous variants in JPH2 (p.T237A/p.I414L), and 3 non-synonymous heterozygous variants in TTN (p.K6609N), DSP (p.R1341H), and MAP2K2 (p.S306I) (Supplemental Table I). At first, we excluded the 3 variants in TTN, DSP, and MAP2K2 from candidate variants for the following reasons. TTN, which is the gene encoding titin, has been implicated in cardiomyopathy. Titin is the largest human protein and is composed of approximately 33,000 amino acids. A recent population screening study showed that rare missense variants were common in TTN and were frequently benign. Herman, et al. reported that every study subject with cardiomyopathy had approximately 1 rare missense variant in TTN, and excluded missense variants from the candidate variants. DSP encodes desmoplakin, which is a primary component of desmosomes. Desmosomes are intercellular adhesion junctions that are most abundant in the epidermis and heart. Although mutations in desmoplakin play a role in DCM, keratoderma, and woolly hair, this patient showed no cutaneous manifestations. MAP2K2 is one of the causative genes of cardio-facio-cutaneous syndrome, which is a multiple congenital anomaly disorder with characteristic craniofacial features, cardiac defects, ectodermal anomalies, neurocognitive delay, and cardiomyopathy. These findings were not found in this case. These 3 genes are inherited in an autosomal dominant manner, but all of the variants were detected in the unaffected father or mother.

We also identified a heterozygous nonsense mutation in PKD1 (p.Q4193*), which was already reported as a confirmed pathogenic mutation in ADPKD. The detected variants in JPH2 and PKD1 were confirmed with Sanger sequencing (Figure 2A, B). Therefore, we speculate that the compound heterozygous variants in JPH2 (p.T237A/p.I414L) were causative variants of DCM, and the heterozygous nonsense mutation in PKD1 (p.Q4193*) may have
been associated with progression of DCM in the current case. 

**Family history:** The patient’s mother was diagnosed with PKD after her daughter’s diagnosis of PKD and the patient’s maternal grandmother had PKD (Figure 2C). The maternal younger brother was diagnosed with a cerebral aneurysm at 30 years old and the maternal grandmother had a subarachnoid hemorrhage at 49 years old. The patient’s father had bronchitis/asthma, but no kidney disease. An echocardiogram of the patient’s parents showed no abnormality.

**Discussion**

In this case, compound heterozygous variants (p.T237A/p.I414L) in *JPH2* were detected. Junctophilin-2 has 8 membrane occupation and recognition nexus domains, including the joining region, α-helical domain, divergent region, and sarcoplasmic reticulum transmembrane domains (Figure 2D). The Thr237 residue is located between membrane occupation and recognition nexus domains and the Ile414 residue is located in the α-helical domain. Few studies have focused on domain-specific functions. Vanninen, *et al* reviewed 19 previously described *JPH2* variants, of which p.E85K, p.S241R, p.N409K, p.Q428*, and p.R522W are associated with DCM, and p.A189T and p.E338G are associated with sudden death. Beavers, *et al* reported that a specific mutation (p.E169K) was associated with juvenile-onset paroxysmal atrial fibrillation. The variants in this case have not been reported. Therefore, the significance of these variants is unknown. These variants are extremely rare (Supplemental Table I), and both Thr237 and Ile414 residues are conserved between species (Figure 2E). These findings suggest that the variants in this case may have been associated with DCM.

Some studies using animal models have suggested a role for junctophilin-2 in the development of cardiomyopathy, with loss of normal junctophilin-2 expression during pathological remodeling. Conventional knock-out of *Jph2* shows embryonic lethality at embryonic day 10.5. 

Cardiac-specific *Jph2* knockdown results in impaired cardiac contractility, and shared congestive heart failure between postnatal days 10 and 15, suggesting that *Jph2* deficiency leads to postnatal heart failure. 

In the neonatal period, the junctional membrane complex including transverse tubules is poorly developed and this transverse tubule maturation is partly governed by junctophilin-2. Therefore, *JPH2* has an important role in structural and functional development in the embryonic and neonatal periods, and patients who possess bi-allelic loss of function *JPH2* variants might show cardiomyopathy in infancy. Slight noncompaction of the left ventricular myocardium in the neonatal period in this case may have been the result of bi-allelic loss of function of *JPH2*.

Previous studies have described that heterozygous *JPH2* variants cause adult-onset DCM/hypertrophic cardiomyopathy, while homozygous nonsense *JPH2* variants cause infantile-onset DCM. Both parents with a heterozygous missense variant (p.T237A or p.I414L) were asymptomatic, suggesting that these variants may be pathogenic loss of function variants in an autosomal recessive manner.

ADPKD is the most common hereditary kidney disorder and is most commonly caused by mutations in the *PKD1* or *PKD2* genes. Cardiovascular problems are a major cause of morbidity and a leading cause of mortality in patients with ADPKD. Cases of hypertrophic cardiomyopathy, DCM, and left ventricular noncompaction with ADPKD have been previously reported (Supplemental Table II). Young patients with ADPKD and normal blood pressure and renal function show early vascular changes and bi-ventricular diastolic dysfunction. This finding suggests that cardiovascular problems with ADPKD are genetic consequences, as well as a secondary to hypertension or renal dysfunction.

Interestingly, PCs are also expressed in endothelial and vascular smooth muscle cells and cardiomyocytes, thus leading to aneurysm and cardiac myopathy. PC1 functions as a mechanosensor that regulates L-type calcium channels in cardiomyocytes. In an experimental study, *Pkd1* null mouse embryos died at embryonic days 13.5-14.5 from a primary cardiovascular defect. In another experimental study, *Pkd2*-mutant zebrafish showed impaired intracellular calcium cycling and heart failure with reduced cardiac output. In this case, the mother with the same heterozygous *PKD1* variant showed no finding of cardiomyopathy, which indicates that the *PKD1* variant is a predisposing factor rather than a direct cause of cardiomyopathy. Interestingly, *PKD1* and *JPH2* are responsible genes for CICR. Therefore, the *PKD1* variant may have accelerated the onset and progression of DCM through impaired calcium cycling in cardiomyocytes in this case.

**Conclusion**

We report a sudden unexpected death of an 8-month old infant with DCM who had *JPH2* variants. She also had PKD with a *PKD1* variant. Interestingly, both of these genes are associated with calcium signaling in the heart. We propose that heterozygous variants in *JPH2* (p.T237A/ p.I414L) lead to protein dysfunction and cause DCM in an autosomal recessive manner.

**Acknowledgment**

We thank J. Narita, H. Ishida and J. Tanigawa of Osaka University for their fruitful discussions.

**Disclosure**

**Conflicts of interest:** The authors have no conflicts of interest to declare.

**References**

1. Soares P, Rocha G, Pissarra H, *et al*. Neonatal dilated cardiomyopathy. Rev Port Cardiol 2017; 36: 201-14.
2. Takeshima H, Komazaki S, Nishi M, Iino M, Kangawa K. Junctophilins: A novel family of junctional membrane complex pro-
teins. Molecular Cell 2000; 6: 11-22.
3. Beavers DL, Landstrom AP, Chiang DY, Wehrens XH. Emerging roles of junctophilin-2 in the heart and implications for cardiac diseases. Cardiovasc Res 2014; 103: 198-205.
4. Sabater-Molina M, Navarro M, Garcia-Molina Saez E, et al. Mutation in JPH2 cause dilated cardiomyopathy. Clin Genet 2016; 90: 468-9.
5. Vanninen SUM, Leivo K, Seppala EH, et al. Heterozygous junctophilin-2 (JPH2) p.(Thr161Lys) is a nonogenic cause for HCM with heart failure. PLoS One 2018; 13: e.0203422.
6. Vasilescu C, Ojala TH, Brilhante V, et al. Genetic Basis of Severe Childhood-Onset Cardiomyopathies. JACC 2018; 72: 2324-38.
7. Jones EG, Mazaheri N, Maroofian R, et al. Analysis of enriched rare variants in JPH2-encoded junctophilin-2 among Greater Middle Eastern individuals reveals a novel homozygous variant associated with neonatal dilated cardiomyopathy. Sci Rep 2019; 9: 9038.
8. Cornec-Le Gall E, Alam A, Perrone RD. Autosomal dominant polycystic kidney disease. Lancet 2019; 393: 919-35.
9. Kuo IV, KwaczaLA AT, Nguyen L, Russell KS, Campbell SG, Ehrlich BE. Decreased polycystin 2 expression alters calcium-contraction coupling and changes beta-adrenergic signaling pathways. Proc Natl Acad Sci U S A 2014; 111: 16604-9.
10. Malekshahabi T, Khoshdel Rad N, Serra AL, Moghadasali R. Autosomal dominant polycystic kidney disease: Disrupted pathways and potential therapeutic interventions. J Cell Physiol 2019; 234: 12451-70.
11. Chebib FT, Hogan MC, El-Zoghby ZM, et al. Autosomal Dominant Polycystic Kidney Patients May Be Predispersed to Various Cardiomyopathies. Kidney Int Rep 2017; 2: 913-23.
12. Suwa Y, Higo S, Nakamoto K, et al. Old-Age Onset Progressive Cardiac Contractile Dysfunction in a Patient with Polycystic Kidney Disease Harboring a PKD1 Frameshift Mutation. Int Heart J 2019; 60: 220-5.
13. Morita H. Secondary Cardiomyopathy in Polycystic Kidney Disease Syndrome. Int Heart J 2019; 60: 10-1.
14. Haas J, Frese KS, Peil B, et al. Atlas of the clinical genetics of human dilated cardiomyopathy. European Heart Journal 2015; 36: 1123-35.
15. Oshima Y, Yamamoto T, Ishikawa T, et al. Postmortem genetic analysis of sudden unexpected death in infancy: neonatal genetic screening may enable the prevention of sudden infant death. J Hum Genet 2017; 62: 989-95.
16. Gigli M, Begay RL, Morea G, et al. A Review of the Giant Protein Titin in Clinical Molecular Diagnostics of Cardiomyopathies. Front Cardiovasc Med 2016; 3: 21.
17. Herman DS, Lam L, Taylor MR, et al. Truncations of titin causing dilated cardiomyopathy. N Engl J Med 2012; 366: 619-28.
18. Boyden LM, Kam CY, Hernández-Martín A, et al. Dominant de novo DSP mutations cause erythrodermatodermia-cardiomyopathy syndrome. Hum Mol Genet 2016; 25: 348-57.
19. Schulz AL, Albrecth B, van der Burgt I, et al. Mutation and phenotypic spectrum in patients with cardio-facio-cutaneous and Costello syndrome. Clin Genet 2008; 73: 62-70.
20. Jin M, Xie Y, Chen Z, et al. System analysis of gene mutations and clinical phenotype in Chinese patients with autosomal-dominant polycystic kidney disease. Sci Rep 2016; 6: 35945.
21. Beavers DL, Wang W, Ather S, et al. Mutation E169K in junctophilin-2 causes atrial fibrillation due to impaired RyR2 stabilization. J Am Coll Cardiol 2013; 62: 2010-9.
22. Minamisawa S, Oshikawa J, Takeshima H, et al. Junctophilin type 2 is associated with caveolin-3 and is down-regulated in the hypertrophic and dilated cardiomyopathies. Biochem Biophys. Res Commun 2004; 325: 852-6.
23. Wei S, Guo A, Chen B, et al. T-tubule remodeling during transition from hypertrophy to heart failure. Circ Res 2010; 107: 520-31.
24. Landstrom AP, Kellen CA, Dixit SS, et al. Junctophilin-2 expression silencing causes cardiocyte hypertrophy and abnormal intracellular calcium-handling. Circ Heart Fail 2011; 4: 214-23.
25. van Oort RJ, Garbino A, Wang W, et al. Disrupted junctional membrane complexes and hyperactive ryanodine receptors after acute junctophilin knockdown in mice. Circulation 2011; 123: 979-88.
26. Reynolds JQ, Chiang DY, Wang W, et al. Junctophilin-2 is necessary for T-tubule maturation during mouse heart development. Cardiovasc Res 2013; 100: 44-53.
27. Chen B, Guo A, Zhang C, et al. Critical roles of junctophilin-2 in T-tubule and excitation-contraction coupling maturation during postnatal development. Cardiovasc Res 2013; 100: 54-62.
28. Lubrano R, Versacci P, Guido G, Bellelli E, Andreoli G, Elli M. Might there be an association between polycystic kidney disease and noncompaction of the ventricular myocardium? Nephrol Dial Transplant 2009; 24: 3884-6.
29. Oflaz H, Alisir S, Buyukaydin B, et al. Biventricular diastolic dysfunction in patients with autosomal-dominant polycystic kidney disease. Kidney Int 2005; 68: 2244-9.
30. Pedrozzi Z, Cristiolo A, Battiprolu PK, et al. Polycystin-1 Is a Cardiomyocyte Mechanosensor That Governs L-Type Ca2+ Channel Protein Stability. Circulation 2015; 131: 2131-42.
31. Bouther C, Mutoy S, Webb S, Fleming S, Brindle K, Sandford R. Cardiovascular, skeletal, and renal defects in mice with a targeted disruption of the Pkd1 gene. Proc Natl Acad Sci U S A 2001; 98: 12174-9.
32. Paavola J, Schliffke S, Rossetti S, et al. Polycystin-2 mutations lead to impaired calcium cycling in the heart and predispose to dilated cardiomyopathy. J Mol Cell Cardiol 2013; 58: 199-208.

Supplemental Files
Supplemental Tables I, II
Please see supplemental files; https://doi.org/10.1536/ihj.20-155