Congenital Heart Disease

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CASE REPORT

Compound Heterozygous *PLD1* Variants in Right-Sided Heart Malformations

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ABSTRACT

We report a three-year-old male child who presented with congenital valvular defects, right ventricular malformation, and initial developmental delay. Genome sequencing showed rare deleterious biallelic missense variants in *PLD1*. In his parents' second pregnancy, echocardiogram at 13 weeks gestation revealed right-sided cardiac malformations resembling the clinical presentation of the family's first child. Targeted DNA analysis showed that the fetus carried the same biallelic *PLD1* variants as their older sibling. This case helps to further delineate the clinical spectrum of *PLD1*-related defects and highlights the value of both genome sequencing in congenital heart disease and early fetal echocardiography to establish phenotype.

KEYWORDS

Genome sequencing; *PLD1*; fetal echocardiogram; right ventricular malformation; congenital valve defects

Nomenclature

ACMG	American College of Medical Genetics and Genomics
ASD	Atrial septal defect
CADD	Combined Annotation Dependent Depletion
CHD	Congenital heart disease
CLIA	Clinical Laboratory Improvement Amendments
CVS	Chorionic villi sample

GnomAD	The Genome Aggregation Database
GS	Genome sequencing
NT	Nuchal translucency
PDA	Patent ductus arteriosus
PolyPhen-2	Polymorphism Phenotyping v2
PLD1	Phosphatidylcholine-specific Phospholipase D1
RV	Right ventricle
SIFT	Sorting Intolerant from Tolerant
TOP	Termination of pregnancy
VSD	Ventricular septal defect

1 Introduction

Congenital heart diseases account for almost one-third of all major congenital anomalies, with a prevalence of 7 per 1000 births [1]. While advances in sequencing efforts have enabled the identification of novel causative CHD-gene associations, the molecular etiology for most CHD remains largely unknown, with the majority being multifactorial. Biallelic variants in *PLD1* have recently been identified in patients with non-syndromic congenital valve defects and RV malformations [2,3]. To our knowledge, only a few studies on CHD have described the use of genomic investigation to identify a single causative gene in a proband and early fetal echocardiography to facilitate a diagnosis in their fetal sibling. Here we report a family with a three-year-old male child and a fetal sibling, diagnosed at 13 weeks gestation, with compound heterozygous variants in *PLD1*.

2 Paediatric Case

The proband was the product of the first pregnancy of a healthy non-consanguineous couple of European descent. A fetal echocardiogram at 20 weeks 6 days gestation done on the mother showed severe pulmonary valve stenosis, hypoplastic tricuspid valve, large muscular VSD, bilateral hypoplastic main pulmonary arteries and critically hypoplastic and dysmorphic RV (multilobular parts) (26-week fetal echocardiogram, Fig. 1A, and supplementary video S1).

Delivery was at term and uncomplicated. The patient's birthweight was 2.85 kg (3–10th centile). Postnatal echocardiography and MRI (Figs. 1B and 1C) confirmed the antenatal diagnosis with the addition of coronary sinusoids (RV) and an aberrant right subclavian artery with left aortic arch.

A univentricular surgical strategy was indicated. At four days of life, the patient underwent a central Blalock-Taussing shunt and main pulmonary artery ligation. At five months, the patient underwent his second stage of repair which included a bidirectional cavopulmonary connection, and takedown of the central shunt. The patient is currently being followed in cardiology and has recently undergone Fontan surgery.

At last assessment at 18 months of age, neurodevelopmental evaluation using the Bayley Scales of Infant and Toddler Development, third edition [4], showed that his receptive language was at the 5th percentile, expressive language was at the 2nd percentile, and his cognitive skills were at the 16th percentile.

Given his complex CHD, the proband was referred for trio GS at the Cardiac Genome Clinic. The analytic workflow was previously described [5]. GS of the proband and his parents showed compound heterozygous missense variants in *PLD1* (NM_002662.5): paternally inherited c.2681A>C, p.(Tyr894Ser), and maternally inherited c.1982A>T, p.(Asp661Val). The variants are present in gnomAD [6], with a European minor allele frequency of 0.017% and 0.01%, respectively, and neither variant is seen in the homozygous state. *In-silico* prediction tools, including CADD [7], PolyPhen-2 [8], SIFT [9],

and ClinPred [10], support a deleterious effect for both variants. The CADD scores for p.(Tyr894Ser) and p.(Asp661Val) are 25.9 and 32, respectively. Both variants lie in the catalytic domain of the protein [3].

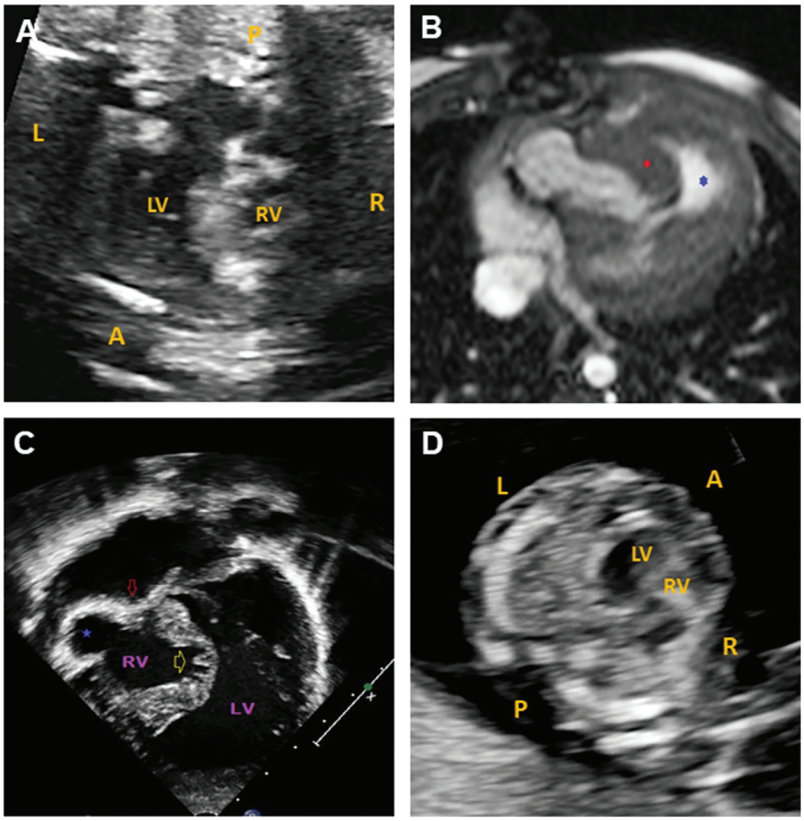

Figure 1: Cardiac imaging for the paediatric case and fetal sibling. (A) 26-week fetal echocardiogram for the paediatric patient showing a hypoplastic RV. (B) Cardiac MRI for the paediatric patient at birth. Axial balanced steady state gradient echo (TRUFISP). Severe tricuspid valve stenosis with RV anterior free wall ballooning (blue star) and hypertrophied moderator band (red diamond). (C) Echocardiography for the paediatric patient at birth. Apical view with severe tricuspid stenosis (red arrow), RV anterior free wall ballooning (blue star), and atrophic and trabecular inter-ventricular septum. (D) 13-week fetal echocardiogram showing a hypoplastic RV in the fetal sibling. L: left, R: right, A: anterior, P: posterior, LV: left ventricle, RV: right ventricle

The paternally inherited missense variant, p.(Tyr894Ser), was previously described in two, unrelated deceased patients from Lahrouchi et al. [3] (Table 1), both of whom harboured the variant in trans with a second variant that severely reduced the catalytic activity of *PLD1* on functional assessment: c.2098C>T, p.(Arg700Cys) and c.1062G>A, p.(Trp354Ter), respectively.

In the proband, clinical validation of the variants was performed in a CLIA-approved laboratory using targeted variant testing. GS did not reveal any additional genomic findings thought to contribute to the patient's phenotype. The family was counselled regarding the 25% recurrence risk for future pregnancies.

Table 1: Clinical comparison of the proband and fetal sibling with reported patients with the c.2681A>C, p.(Tyr894Ser) *PLD1* variant from Lahrouchi et al. [3]

Patient ID	Age	*PLD1* genotype	Right-sided valvular defects	RV defect	Other cardiac defects	Extracardiac features
Paediatric case	3 years	c.2681A>C; c.1982A>T, p.(Tyr894Ser); p.(Asp661Val)	Hypoplastic tricuspid valve, pulmonary valve stenosis	Hypoplastic and dysmorphic	VSD, hypoplastic pulmonary arteries, aberrant right subclavian artery, left aortic arch coronary sinusoids	Initial developmental delay
Fetal sibling	13 weeks, 3 days gestation (TOP)	c.2681A>C; c.1982A>T, p.(Tyr894Ser); p.(Asp661Val)	Hypoplastic tricuspid valve	Hypoplastic		
Family P I-1 [3]	23 days (deceased)	c.2681A>C; c.2098C>T, p.(Tyr894Ser); p.(Arg700Cys)	Tricuspid valve atresia, absent pulmonary valve	Hypoplastic	Mitral valve regurgitation, ASD, left ventricular hypertrophy, tortuous PDA, ectopic atrial tachycardia, mildly decreased systolic function	5th finger clinodactyly, large earlobes, diffuse anasarca, acute respiratory failure with necrotizing pneumonia and left pneumothorax, small right temporal hemorrhage
Family Q I-1 [3]	16 weeks gestation (fetal demise)	c.2681A>C; c.1062G>A, p.(Tyr894Ser); p.(Trp354Ter)	Tricuspid valve atresia, pulmonary valve atresia	Hypoplastic		

3 Fetal Sibling

The couple's second pregnancy was naturally conceived and NT ultrasound at 12 weeks gestation was 3.1 mm. Enhanced first trimester screening done subsequently showed an increased risk for Trisomy 21 (1 in 12 at term). Given the history of a child with complex CHD, the family was referred for fetal ultrasound and echocardiography at 13 weeks gestation. The fetal imaging showed hypoplastic, non-apex forming RV and hypoplastic tricuspid valve with minimal, monophasic inflow (Fig. 1D and supplementary videos S2–S4). No VSD was present. Normal left-sided structures were observed.

The right-sided valvular defects with hypoplastic RV had marked overlap with the cardiac features of the family's three-year-old son. Following counselling, the couple chose to terminate the pregnancy. Targeted DNA analysis for the familial *PLD1* gene showed that the fetus had the same compound heterozygous variants as their son. Microarray analysis done on CVS was normal.

Using the ACMG criteria [11], the added segregation data from this family enabled a classification of Likely Pathogenic for the previously reported p.(Tyr894Ser) variant (PM2, PM3, PP3, PP1_Moderate [12]), and a classification of Variant of Uncertain Significance for the p.(Asp661Val) variant (PM2, PP1, PP3).

4 Discussion

Phosphatidylcholine-specific Phospholipase D1 (*PLD1*) catalyzes the hydrolysis of phosphatidylcholine to produce phosphatidic acid and choline. *PLD1* has been implicated in numerous cellular pathways, including membrane trafficking, signal transduction, and cytoskeleton organization [13]. Recent research revealed that loss of *PLD1* function in a recessive manner results in CHD [2,3]. Cardiac defects caused by loss of *PLD1* generally led to abnormal development of the cardiac valves on the right chamber.

The advancement of next-generation sequencing technologies and computational strategies is accelerating the rate of novel gene discovery for CHD, and newly associated rare disorders associated with CHD are continuously identified [14–16]. Given this rapid gene discovery and the rarity of these disorders, panels and targeted testing are impractical, making GS essential for identifying cases with high recurrence risk. In the presented case, GS was critical in providing the family with a clear diagnosis for their three-year-old child and allowed us to appropriately counsel the family on their 25% recurrence risk in future pregnancies.

Evaluation of the heart in the fetal sibling enabled early detection of the cardiac abnormality and genetic diagnosis of the *PLD1*-related cardiac lesions. The specific clinical manifestation of *PLD1*-related CHD was identified on echocardiography early in the pregnancy and was beneficial for the family in decision making.

The family's added segregation data allowed an upgraded classification of Likely Pathogenic for the previously reported p.(Tyr894Ser) variant. Patients reported to have the p.(Tyr894Ser) variant present with a striking phenotypic overlap, consisting of tricuspid valve and pulmonary valve anomalies, and hypoplastic RV [3]. The three-year-old proband is the only reported survivor with the p.(Tyr894Ser) variant.

The proband also presented with initial developmental delay. While developmental concerns are not explicitly described for other *PLD1* patients, the sample size of patients who survived past infancy is small. Additional cases are needed to further delineate the clinical spectrum of *PLD1*-related defects and the possible association of *PLD1* with developmental delay.

5 Conclusion

This family shows the importance of GS in identifying the causative gene in CHD patients and the importance of fetal echocardiography to establish the phenotype. The value of GS for this family extends to patient populations with rare disorders, such as this one, with high recurrence risks. In the context of an accurate recurrence risk, molecular diagnosis through GS can enable preimplantation and prenatal diagnosis, and early fetal echocardiography, at 12–13 weeks gestation, can accurately identify the cardiac abnormalities.

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Ethics Approval: The study was approved by the Hospital for Sick Children (REB 1000053844).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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