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Novel Homozygous Large Deletion Mutation in the *PKLR* Causes Transfusion-Dependent Severe Pyruvate Kinase Deficiency: A Case Report

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ABSTRACT Pyruvate kinase deficiency (PKD) is an autosomal recessive erythrocytic enzymopathy driven by biallelic PKLR defects, classically presenting as congenital non-spherocytic haemolytic anaemia. This report details the molecular and clinical characterisation of a previously unannotated homozygous structural rearrangement affecting PKLR. The proband, a 7-month-old male with sustained transfusion dependence since birth, underwent targeted sequencing coupled with multiplex copy-number variation profiling, which identified a complete homozygous excision spanning exons 3 to 9 (NM_000298.6). Parental genotyping confirmed trans-inheritance of this allele from each heterozygous carrier. This ~70-kb genomic deletion, removing seven coding exons, represents a mutational mechanism absent from current PKD variant databases. Currently, the patient remains transfusion-committed and is scheduled for allogeneic haematopoietic stem cell engraftment. This case unequivocally demonstrates that comprehensive deletion-detection assays are non-negotiable for accurate PKD subtyping, as standard exon-limited panels would miss this lesion, thereby directly informing prognosis, transplantation timing, and cascade carrier screening across affected kindreds

INTRODUCTION

Pyruvate kinase deficiency (PKD) is the most common inherited enzymopathy of the erythrocyte glycolytic pathway, characterised by chronic hemolytic anemia transmitted in an autosomal recessive manner (Grace et al. 2015). Its hallmark is chronic non-spherocytic haemolytic anaemia, which is often accompanied by complications such as hyperbilirubinemia, hepatosplenomegaly, cholelithiasis, and iron overload due to chronic hemolysis and transfusional burden (Barcellini and Fattizzo 2015; Al-Samkari et al. 2020).

Because mature erythrocytes are devoid of mitochondria, they depend exclusively on glycolysis for adenosine triphosphate (ATP) production. Within this metabolic route, pyruvate kinase (PK) acts as a principal rate-controlling enzyme, mediating the terminal step in which phosphoenolpyruvate is converted to pyruvate alongside the irreversible transfer of a phosphate group to ADP (Bianchi et al. 2019; Rafat et al. 2026). Any impairment in PK activity interrupts this essential ATP-generating reaction. The resultant decline in ATP levels subsequently undermines the operation of membrane-bound ion pumps that rely on this energy source, specifically Na^+ /K^+ -ATPase and Ca^{2+} -ATPase. This pump dysfunction triggers a cascade of cellular alterations, including water loss, increased membrane stiffness, and eventual destruction of erythrocytes by extravascular phagocytosis, predominantly occurring within the spleen

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and liver (Roy et al. 2021; Traets et al. 2025). At the same time, this enzymatic bottleneck causes the build-up of precursor glycolytic metabolites, most notably 2,3-diphosphoglycerate (2,3-DPG). Elevated 2,3-DPG levels diminish haemoglobin's oxygen-binding capacity, inducing a rightward displacement of the oxygen-hemoglobin dissociation curve. This adaptive shift may partially offset tissue hypoxic stress, even in the presence of reduced circulating red cell mass (Grace et al. 2019).

In human physiology, four distinct PK isoforms originate from two separate genetic loci, that is, the PKM gene gives rise to the PKM1 and PKM2 variants, which are prevalent in skeletal muscle, neural tissue, leukocytes, and actively dividing cells, whereas the PKLR gene encodes both the hepatic (PKL) and erythroid (PKR) isozymes (Noguchi et al. 1986; Valentini et al. 2002, Alquraishi et al. 2019; Puckett et al. 2021). PKD arises exclusively from biallelic pathogenic mutations affecting the PKLR gene, which ultimately compromise either the structural integrity or the enzymatic turnover capacity of the PKR protein. These genetic defects frequently alter the enzyme's binding affinity toward its primary substrate (phosphoenolpyruvate) or modify its responsiveness to the allosteric activator fructose-1,6-bisphosphate (Bianchi et al. 2020).

PKD has an estimated global prevalence of 3 to 8 per million, though it may be underdiagnosed (Atkinson et al. 2025). Over 350 pathogenic variants in *PKLR* have been documented, encompassing missense, nonsense, splicing variants, and small to large insertions/deletions, contributing to a broad phenotypic spectrum (Wang et al. 2024). The genotype-phenotype correlation, while complex, is crucial for understanding disease variability and guiding management, which ranges from supportive care to novel pharmacotherapy with the recently approved allosteric activator mitapivat, or definitive cure via haematopoietic stem cell transplantation (van Straaten et al. 2018; Al-Samkari et al. 2022; Qin et al. 2025). This case contributes to the growing literature on the genotypic diversity of PKD and its clinical implications.

Objectives

The principal aim of this report is to characterise a previously unrecorded germline homozygous structural variant involving the *PKLR*, which results in the contiguous deletion of coding exons 3 through 9. This specific mutational architecture has not been annotated in existing genomic databases nor described in peer-reviewed literature to date, thereby constituting a distinct addition to the allelic heterogeneity of PKD.

MATERIAL AND METHODS

Clinical Data Collection and Physical Examination

This report was prepared with approval from the institutional Medical Ethics Committee of Shunde Women and Children's Hospital of Guangdong Medical University and written informed consent from the patient's guardians. A retrospective, in-depth analysis was performed on a single patient (7-month-old male infant) admitted to Shunde Women and Children's Hospital, Guangdong Medical University, Foshan, China, for the evaluation and management of persistent anaemia (>6 months duration) and acute worsening marked by pallor over the preceding week. His clinical course was characterised by severe, transfusion-dependent congenital haemolytic anaemia. Data collected from the patient and guardians, included a detailed assessment of the patient's baseline demographics, such as age, sex, weight, height, and body mass index. The nature, duration, and progression of presenting symptoms were documented, with particular attention to altered consciousness and behavioural changes. A thorough past medical history was obtained, focusing on previous similar episodes, documented dietary habits with an emphasis on protein aversion, developmental milestones, academic performance, and prior medication use. A detailed family history was elicited, specifically probing for neurological, metabolic, or unexplained illnesses in first- and second-degree relatives.

Diagnostic Laboratory Procedures and Specimen Handling

All diagnostic specimens (blood, serum, plasma, urine) were collected, processed, and analysed. For the initial metabolic and biochemical work-up, venous draws were obtained using the designated vacuum tube collection systems. The pa-

tient underwent a complete blood count (CBC) on a fortnightly basis, with all measurements carried out via an automated cell counter. The panel of haematological variables tracked during this monitoring period encompassed total leukocyte and erythrocyte counts, haemoglobin concentrations (recorded both immediately prior to and following each transfusion), haematocrit percentages, standard erythrocyte indices (namely MCV, MCH, and MCHC), as well as platelet numbers, procalcitonin levels, and the relative reticulocyte fraction. Finally, all obtained clinical readings were benchmarked against established normative reference intervals specifically stratified for the patient's chronological age group.

Initial Biochemical and Disease-Specific Screening

To rule out common causes of anaemia and haemolysis, a series of targeted tests were conducted as follows.

Haemolysis and Blood Disorder Workup

The patient underwent an ABO haemolysis test, glucose-6-phosphate dehydrogenase (G6PD) enzyme activity quantification, and a methemoglobin reduction test. A direct Coombs test and irregular antibody screening were performed to rule out immune-mediated hemolysis. A red blood cell fragility test was performed to assess for hereditary spherocytosis.

Haemoglobinopathy Screening

Haemoglobin (HGB) electrophoresis was performed when the patient was 2 months old to quantify the levels of Haemoglobin A (HbA), Haemoglobin A2 (HbA2), and Haemoglobin F (HbF). Subsequently, a comprehensive screening for common thalassaemia-related gene mutations was conducted.

Nutritional and Metabolic Assessment

Serum levels of vitamin B12, folate, and ferritin were measured. Total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), and total bile acids were measured to assess haemolysis and liver function. Genetic metabolic screening and haemophilia factor screening (including von Willebrand factor) were also performed.

Bone Marrow Examination

A bone marrow aspiration was performed. The aspirate was analysed by flow cytometry to rule out malignant diseases such as leukaemia and myelodysplastic syndromes.

Advanced Genetic Analysis

Whole Exome Sequencing (WES)

To identify the underlying genetic etiology, genomic DNA was extracted from 2 mL of peripheral blood collected from the patient and his parents. Whole exome sequencing was performed on these samples. Sequence data were analysed to identify potential disease-causing variants, with a focus on genes associated with immune system and haematological disorders.

Next-Generation Sequencing (NGS) and Familial Verification

Following the identification of a suspected pathogenic mutation in the PKLR gene via WES, targeted next-generation sequencing was conducted on all family members (patient, parents, and sibling) for validation.

Copy Number Variation (CNV) Analysis

Fluorescence quantitative PCR (qPCR) was employed to test for deletion or duplication events within the PKLR gene segments across the family. This method was used to confirm copy number variations.

Semi-quantitative and Quantitative PCR

Semi-quantitative PCR was performed to confirm the biallelic deletion in the proband and monoallelic carrier status in the parents and sibling. Quantitative dosage analysis was carried out to determine the copy number of specific exons, measuring the reduction in heterozygous carriers and the absence of an amplifiable product in the proband.

Bioinformatic and Pathogenicity Analysis

The identified genetic alteration was cross-referenced against major variant databases, includ-

ing ClinVar, HGMD, OMIM, gnomAD, ExAC Browser, and the 1000 Genomes Project. In silico prediction tools, namely Polyphen-2, the Ensembl Variant Effect Predictor (VEP), and SIFT, were used to predict the variant's impact on protein structure and function. The clinical significance of the variant was classified according to the American College of Medical Genetics and Genomics (ACMG) 2015 guidelines (Lee et al. 2025).

RESULTS

Perinatal and Neonatal Course

The patient presented in the immediate neonatal period with respiratory distress, cyanosis, and hyperbilirubinemia. Initial evaluation led to diagnoses of neonatal septic shock, anaemia, and hyperbilirubinemia. He was managed with blood transfusion, intensive phototherapy, and broad-spectrum antimicrobial therapy, with subsequent clinical stabilisation. Although the profound anaemia was initially attributed to infection-induced hemolysis, a concurrent congenital etiology was suspected but not fully investigated.

Clinical Trajectory and Management

Over the ensuing seven months, the patient demonstrated a chronic, severe haemolytic phenotype, requiring 15 packed red blood cell transfusions across multiple hospital admissions. His anaemia exhibited marked instability, with rapid clinical decompensation during intercurrent infections. His transfusion regimen consisted of approximately 0.5 units of leukocyte-reduced packed red blood cells every two weeks to maintain haemodynamic stability and mitigate complications of severe chronic anaemia.

Personal and Family History

The patient is the second live-born child of nonconsanguineous and healthy parents. He was delivered by cesarean section at 39-week gestation due to foetal distress. The family history was negative for inherent diseases.

Physical Examination

Physical examination revealed pale skin throughout the body, yellowish sclera, normal cardiovascular and pulmonary findings, and no enlargement of the liver or spleen.

Laboratory Examinations

Since the first admission 7 months ago, the researchers have been actively searching for the cause of anaemia in this child. Routine blood tests were regularly performed every 2 weeks, and the results listed in Table 1.

Moreover, the Blood Group System (ABO) haemolysis test and glucose-6-phosphate dehydrogenase (G6PD) enzyme activity were normal, and diseases such as ABO haemolysis and G6PD could be excluded. HGB electrophoresis was performed when the patient was aged 2 months, and

Table 1: Postnatal complete blood counts

Items	*Values*	*Age-matched normal range*
WBCs (10^9/L)	4.22-16.57	4-10
RBCs (10^{12}/L)	1.75-4.41	4.3-5.8
Pre-transfusion HGB (g/L)	58.4-89	110-160
Post-transfusion HGB (g/L)	99-124	170-200
HCT	0.17-0.39	0.4-0.5
MCV (fL)	83.2-99.1	82-100
MCH (pg)	26.4-33.1	27-34
MCHC (g/L)	307-345	316-354
PLTs (10^9/L)	212-655	125-350
PCT	0.23-0.49	0.14-0.35
Percentage of reticulocytes (%)	0.91-18.49	0.3-3

WBCs: White blood cells; RBCs: Red blood cells; HGB: Haemoglobin; HCT: Haematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; PLT: Platelets; PCT: Procalcitonin

abnormal results were found, with haemoglobin A at 91.8 percent (normal range: 54.5%-75.5%), haemoglobin A2 at 2.3 percent (normal range: 0.5%-1.5%), and haemoglobin F at 2.2 percent to 5.9 percent (normal range: 23%-45%), suggesting a possible diagnosis of thalassaemia. Subsequently, a comprehensive screening for thalassaemia-related genes was conducted, but none was detected.

The regular routine blood tests of the patient showed characteristics of normocytic normochromic anaemia with elevated bilirubin, mainly indirect bilirubin, and the possibility of haemolytic anaemia was considered. Haemolysis-related examinations and bone marrow aspiration were performed. The results of haemolysis-related examinations and flow cytometry of bone marrow cells did not show significant abnormalities (Fig. 1A), ruling out haemolytic anaemia diseases such as G6PD deficiency, hereditary spherocytosis, and paroxysmal cold haemoglobinuria, as well as malignant diseases such as leukaemia and myelodysplastic syndromes. Subsequently, genetic metabolic screening, haemophilia factor screening, and folate and vitamin B12 tests were also conducted, and no significant abnormalities were found, ruling out genetic metabolic diseases, haemophilia, and megaloblastic anaemia (Table 2).

Further Diagnostic Work-up

With family permission, the researchers refined the genetic testing for immune system and blood diseases to help with diagnostic clarification. 2 mL peripheral blood was collected from the patient and his parents. Whole exome sequencing was performed to screen for mutations, and a suspected pathogenic mutation was detected. Subsequently, next-generation sequencing was conducted for familial verification. Using fluorescence quantitative PCR, the researchers tested for deletion/duplications events in gene segments of the family. This method confirmed copy number variations, identifying a novel homozygous large deletion mutation in the *PKLR*, located at exons 3-9. This mutation was inherited from both parents, as depicted in the family pedigree (Fig. 1B). Semi-quantitative PCR confirmed biallelic *PKLR* deletion in the proband, with monoallelic deletions identified in both parents and the sibling, consistent with autosomal recessive inheritance (Fig. 1C). Quantitative dosage analysis of specific exons demonstrated approximately 50 percent reduction in heterozygous carriers and complete absence of amplifiable product in the proband, confirming homozygous loss of the target regions (Fig. 1D). This genomic alteration is absent from all major variant

Table 2: Biochemical and genetic analyses for specific diseases

Items	*Values*	*Age-matched normal range*
TBIL (ìmol/L)	74.9	3.4-26
DBIL (ìmol/L)	21.3	0-7
IBIL (ìmol/L)	45.1	1.7-17
Total bile acids (ìmol/L)	7.66	0-10
HbA (%)	91.8	54.5-75.5
HbA2 (%)	2.3	0.5-1.5
HbF (%)	2.2-5.9	23-45
Vitamin B12 (pg/mL)	761.94-1094.66	180-900
Folate (ng/mL)	> 25	4.2-19.02
Ferritin (ng/mL)	890	30-400
G6PD enzyme activity (U/L)	1782	1700-4000
Methemoglobin reduction test (%)	80	> 75
Von Willebrand factor (%)	133	50-160
Heinz body (%)	8	< 30
Coombs	Negative	Negative
Irregular antibody screening	Negative	Negative
H inclusion bodies	Negative	Negative
G6PD fluorescent spot test	Normal	Normal
Thalassaemia gene testing	Normal	Normal
Haemoglobin electrophoresis	Normal	Normal
Red blood cell fragility test	Normal	Normal

TBIL: Total bilirubin; DBIL: Direct bilirubin; IBIL: Indirect bilirubin; Hb: Haemoglobin; G6PD: Glucose-6-phosphate dehydrogenase

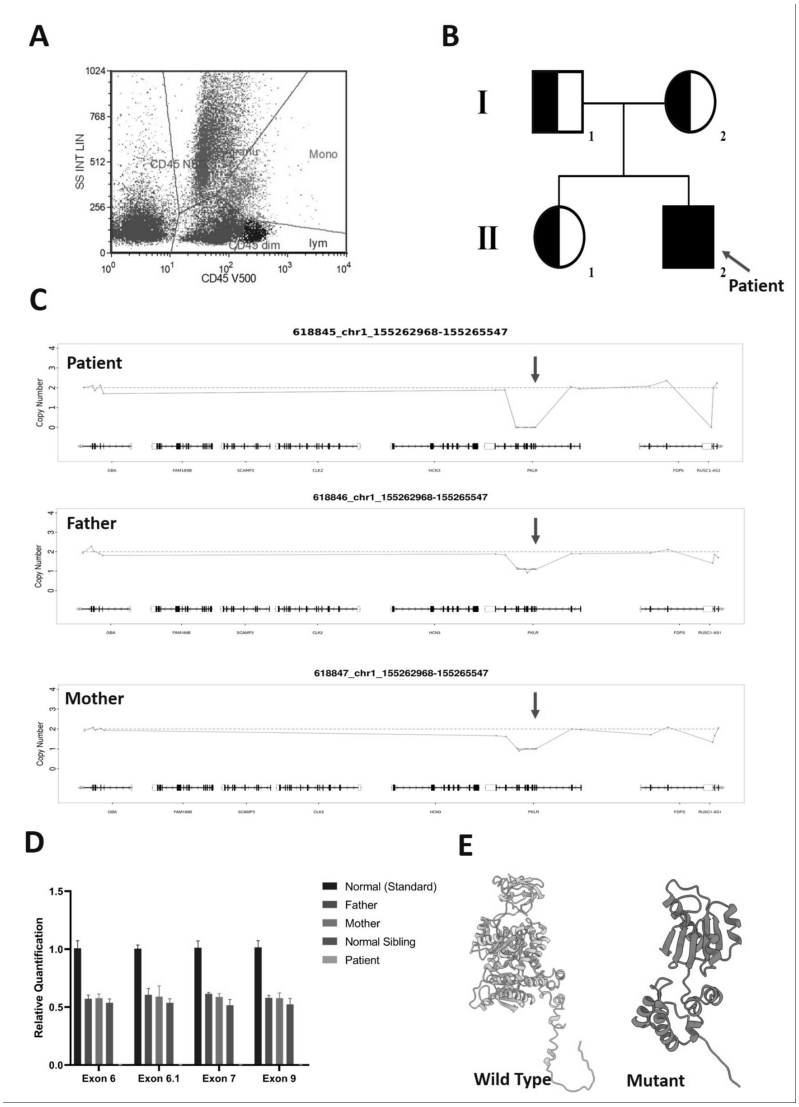

Fig. 1. Clinical, genetic, and molecular characterisation of the *PKLR* deletion

(A) Multiparameter flow cytometry immunophenotyping demonstrates no evidence of acute leukaemia, non-Hodgkin lymphoma, or high-risk myelodysplastic syndrome-associated immunophenotypic aberrancies in the submitted specimen.

(B) Pedigree illustrating autosomal recessive inheritance. The proband (indicated by arrow) is the only affected individual, as parents and siblings are heterozygous carriers.

(C) Copy number variation (CNV) analysis by next-generation sequencing confirms biallelic *PKLR* deletion in the proband and monoallelic deletion in heterozygous carriers. The proband exhibits near-zero copy number, consistent with homozygous deletion. Parents and siblings demonstrate ~50% reduction, confirming heterozygous deletion.

(D) Quantitative polymerase chain reaction (qPCR) validation of *PKLR* exon dosage. Relative quantification of exons 6, 6.1, 7, and 9 is normalised to a reference gene and calibrated against a healthy control (RQ = 1.0). Heterozygous carriers (father, mother, and sibling) exhibit RQ values ~0.5 while the proband shows RQ values approaching zero, confirming homozygous deletion of the target exons.

(E) Structural prediction analysis shows the modifications in the wild type and mutant one

databases, including ClinVar, HGMD, OMIM, and population reference resources (gnomAD, ExAC Browser, 1000 Genomes Project), indicating it represents a novel, previously unreported *PKLR* mutation. In silico prediction tools (Polyphen-2, VEP, SIFT) uniformly classified the variant as pathogenic with predicted deleterious effects on protein structure and function (Fig. 1E). Under the 2015 variant interpretation guidelines established by the American College of Medical Genetics and Genomics (ACMG) (Lee et al. 2025), the observed deletion spanning exons 3 through 9 accumulated sufficient evidence to satisfy three distinct lines of pathogenic criteria. These included PVS1, as this gene is known to operate under a loss-of-function disease model, PM2, given that this alteration is entirely absent from population reference databases, and PP3, which is supported by a convergence of multiple in silico predictive tools all pointing toward a detrimental functional consequence. Taken together, these findings warrant a definitive designation of this genetic alteration as pathogenic.

Final Diagnosis

The patient showed recurrent moderate anaemia, long-term dependence on blood transfusions, and haemolysis. The clinical symptoms of the patient were consistent with PKD. Laboratory examinations excluded common causes of hemolytic anaemia such as G6PD deficiency and thalassaemia. Combined with laboratory and relevant genetic tests, the final diagnosis in this child was PKD.

Treatment

Treatment of this patient focused on supportive and symptomatic care. This includes long-term and regular transfusion of RBCs to maintain adequate HGB levels.

Outcome and Follow-Up

Since PKD diagnosis, the patient has undergone regular transfusions. The parents are now considering haematopoietic stem cell transplantation (HSCT). A comprehensive post-HSCT follow-up includes monitoring initial vital signs, infection, and graft status, addressing midterm graft *vs* host disease (GVHD) prevention/treatment, immune recovery, and metabolic markers, and long-term growth, quality of life, and late complications assessment. The goals of HSCT are successful haematopoietic reconstitution, reduced transfusion needs, and improved quality of life by preventing potential complications (GVHD, infections, etc.). Multidisciplinary care and timely adjustments are required.

DISCUSSION

PKD represents the most common congenital cause of chronic non-spherocytic haemolytic anaemia, with an estimated prevalence ranging from 1:100,000 to 1:300,000 individuals (Al-Samkari et al. 2024). The clinical phenotype exhibits remarkable heterogeneity, spanning from fully compensated hemolysis in asymptomatic individuals to transfusion-dependent anaemia with life-threatening complications (Maciak et al. 2024). This variability poses significant diagnostic and therapeutic challenges, particularly in severely affected patients requiring chronic transfusion support.

Clinical Heterogeneity and Genotype-Phenotype Correlations

The spectrum of PKD severity has traditionally been categorised into mild, moderate, and severe phenotypes based on clinical manifestations and transfusion requirements. Destructive mutations including large deletions, frameshift variants, nonsense mutations, and splicing aberrations have been associated with more severe clinical courses (Canu et al. 2016; Al-Samkari et al. 2024). However, the index case demonstrates an important departure from this paradigm. Despite harbouring a homozygous large deletion of exons 3-9 a mutation predicted to result in complete loss of functional PK enzyme, the patient manifested recurrent moderate anaemia rather than the anticipated life-threatening phenotype. This observation underscores the complex, and still incompletely understood, interplay between genotype and phenotype in PKD. Modifier genes, compensatory metabolic pathways, and individual variations in erythrocyte turnover may all contribute to this disconnect between molecular prediction and clinical expression (Maciak et al. 2024).

Diagnostic Challenges and the Evolving Role of Molecular Analysis

Historically, the diagnosis of PKD has relied upon measurement of erythrocyte PK enzyme ac-

tivity. However, this approach is fraught with limitations. PK activity demonstrates age-dependent variation, with reticulocytes exhibiting significantly higher enzymatic activity than mature erythrocytes. During haemolytic episodes, compensatory reticulocytosis and active bone marrow erythropoiesis introduce large cohorts of young erythrocytes into circulation, potentially normalising or even elevating PK activity measurements despite underlying genetic deficiency. Furthermore, recent transfusions confound enzymatic assessment through the introduction of donor erythrocytes with normal PK activity. These diagnostic pitfalls frequently result in false-negative testing and delayed or missed diagnosis (Maciak et al. 2024).

The ClinGen Prenatal GCEP has recently reaffirmed the definitive gene-disease relationship between *PKLR* and autosomal recessive PKD, with loss-of-function established as the primary pathogenic mechanism. Contemporary genetic diagnostic approaches, including comprehensive sequencing of *PKLR* exons, flanking intronic regions, and promoter sequences, now enable definitive molecular diagnosis in cases where biochemical testing remains inconclusive (Maciak et al. 2024). The identification of the patient's homozygous large deletion mutation, confirmed through parental testing and unreported in existing databases, underscores the critical importance of comprehensive genetic analysis capable of detecting structural variants, which may elude standard sequencing methodologies.

Molecular Characterisation of a Novel *PKLR* Deletion

The *PKLR* resides on chromosome 1q21, encompassing 12 exons that encode the 574-amino acid pyruvate kinase polypeptide (molecular weight 61.8 kDa) (Maciak et al. 2024). Exons 1 and 2 are isoform-specific, directing transcription of the erythrocyte (PKR) and hepatic (PKL) isoforms, respectively, and the remaining ten exons are common to both isoforms. The proband's homozygous deletion spanning exons 3-9 disrupts the shared coding region critical for enzyme function. This structural alteration predictably induces a frameshift during translation of exon 10, leading to premature termination and nonsense-mediated mRNA decay. The resultant absence of functional PK protein explains the severe, transfusion-dependent phenotype observed from the neonatal period. The identification of this identical large deletion in both non-consanguineous parents represents an exceedingly rare molecular event, expanding the known mutational spectrum of *PKLR* and informing genetic counselling for this family.

Contemporary Management Paradigms

The therapeutic landscape for PKD has undergone fundamental transformation with the approval of mitapivat, a first-in-class, oral, allosteric activator of erythrocyte pyruvate kinase (van Beers et al. 2024; Hodroj et al. al. 2025). Unlike traditional supportive measures, mitapivat directly addresses the underlying enzymatic deficiency by binding to and stabilising the active tetrameric conformation of both wild-type and multiple mutant PK enzymes (Hodroj et al. 2025). Pivotal phase III clinical trials have established its efficacy across the disease spectrum. In non-transfusion-dependent adults (ACTIVATE), mitapivat achieved hemoglobin response ($\geq$1.5 g/dL increase sustained at multiple assessments) in 40 percent of patients versus 0 percent with placebo ($p<0.0001$) (van Beers et al. 2024; Hodroj et al. 2025). Among regularly transfused adults (ACTIVATE-T), 37 percent experienced $\geq$33 percent reduction in transfusion burden, with 22 percent achieving transfusion independence (Hodroj et al. 2025).

Notably, recent evidence demonstrates that mitapivat confers additional benefits beyond haemoglobin improvement. Long-term follow-up from the ACTIVATE extension study revealed significant amelioration of ineffective erythropoiesis and iron overload complications once considered inevitable consequences of chronic haemolytic states regardless of transfusion history (van Beers et al. 2024). Patients receiving mitapivat exhibited meaningful improvements in erythroferrone, hepcidin, soluble transferrin receptor, and erythropoietin levels, accompanied by reduction in liver iron concentration at 96 weeks (van Beers et al. 2024). These findings establish mitapivat as the first disease-modifying pharmacotherapy capable of attenuating the pathophysiological cascade linking chronic haemolysis to end-organ iron deposition.

Current Treatment Recommendations

The 2024 International Expert Guidelines for the Diagnosis and Management of Pyruvate Ki-

nase Deficiency, developed using GRADE methodology and the AGREE II framework, provide evidence-based consensus recommendations incorporating these therapeutic advances (Al-Samkari et al. 2024). For symptomatic patients, a graduated approach is recommended, including transfusion support for acute decompensation or chronic severe anaemia, followed by consideration of disease-modifying therapy. In adults ≥18 years, a PK activator trial is now recommended as first-line targeted therapy, with consideration of haematopoietic stem cell transplantation (HSCT) or gene therapy clinical trials in non-responders (Luke et al. 2023; Al-Samkari et al. 2024). For pediatric patients, transfusion support remains the mainstay, with referral for HSCT evaluation and consideration of clinical trial enrollment (Luke et al. 2023).

Allogeneic HSCT remains the sole curative intervention, though experience remains limited and long-term outcomes require further delineation. The procedure carries inherent risks of graft-versus-host disease, infection, and conditioning-related toxicity, necessitating careful risk-benefit assessment in individual patients. Gene therapy approaches, designed to introduce functional *PKLR* transgenes into autologous haematopoietic stem cells, represent an emerging curative strategy currently under clinical investigation (Luke et al. 2023; Al-Samkari et al. 2024). Nutritional interventions, including folate supplementation to support accelerated erythropoiesis, remain important adjunctive measures. Splenectomy, while historically employed, demonstrates variable efficacy and is now reserved for select cases refractory to medical management (Luke et al. 2023). The therapeutic armamentarium for PKD now extends beyond supportive care to include targeted pharmacologic intervention with mitapivat, which demonstrates efficacy in improving haemoglobin, reducing transfusion burden, and ameliorating iron overload. Ongoing investigation of mitapivat in broader hemolytic disease populations including thalassaemia and sickle cell disease suggests its therapeutic potential may extend beyond PKD alone (Guimaraes Barbosa Coelho et al. 2024; Hodroj et al. 2025).

CONCLUSION

This case constitutes the inaugural report of a homozygous contiguous deletion spanning PKLR exons 3-9, confirming that comprehensive copy-number variation profiling is indispensable for definitive diagnosis when standard sequencing approaches prove inconclusive. The severe transfusion-dependent phenotype observed reinforces the complex and multifactorial nature of disease expression, highlighting that clinical trajectory is modulated by genetic and physiological modifiers beyond the primary mutation. Consequently, therapeutic strategies must be individually calibrated to each patient's unique haematologic and metabolic profile.

RECOMMENDATIONS

The researchers advocate for the routine integration of structural variant-detection platforms into diagnostic algorithms for congenital haemolytic anemias to prevent missed diagnoses. Future investigative efforts should prioritise the systematic identification of phenotypic modifiers, rigorous long-term safety monitoring of pyruvate kinase activators in pediatric populations, refinement of conditioning protocols to reduce transplant-related toxicities, and continued advancement of gene-editing modalities as a durable curative alternative to allogeneic stem cell engraftment.

DECLARATIONS

ETHICS APPROVAL AND CONSENT

Written informed consent was obtained from the patient's legal guardian for publication of this case report and any accompanying data.

COMPETING INTERESTS

The authors declare no competing interests.

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