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CASE REPORT

Novel *PKLR* missense mutation (A300P) causing pyruvate kinase deficiency in an Omani Kindred—PK deficiency masquerading as congenital dyserythropoietic anemia

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Abstract

We report herein a child with transfusion-dependent chronic anemia, the cause of which was difficult to establish because of his transfusion dependency. The clinical and laboratory features suggested a chronic nonspherocytic hemolytic anemia (CNSHA) with bone marrow features suggestive of congenital dyserythropoietic anemia (CDA). DNA studies, however, revealed the underlying condition to be due to a novel mutation in the *PKLR* gene responsible for pyruvate kinase deficiency (PKD). Molecular investigations by a targeted next-generation sequencing (t-NGS) using a custom panel of 71 genes involved in the red blood cell (RBC) disorders revealed that the patient was homozygous for a novel missense mutation c.898G>C, p.Ala300Pro, whereas both his parents were heterozygous for the same mutation.

KEYWORDS

congenital dyserythropoietic anemia, hereditary anemia, PKLR missense mutation (A300P), pyruvate kinase, targeted next-generation sequencing

1 | INTRODUCTION

Pyruvate kinase deficiency (PKD) is an inherited autosomal recessive metabolic disorder affecting the enzyme pyruvate kinase leading to the most common glycolytic defect producing a congenital nonspherocytic hemolytic anemia.[1] Pyruvate kinase (PK) converts phosphoenolpyruvate to pyruvate, augmenting the red blood cell (RBC) adenine triphosphate (ATP) production. Since the lifespan of an RBC is dependent on the amount of ATP production by glycolysis, PKD leads to lower ATP production and a reduced RBC survival. The RBCs in PKD are variably damaged with the youngest cells, at highest risk for destruction as they are the most dependent on ATP production by glycolysis, whereas the older red cells are less affected.[2,3] Blockage in the splenic capillaries causes' damage to the affected RBCs, leading to an extravascular hemolysis as these RBCs are destroyed by the reticuloendothelial system both in the spleen and in the liver.

The gene encoding for PK (*PKLR*) has been localized to the long arm of chromosome 1, with the cDNA coding for a 574 amino acids peptide.[4] Approximately 350 different

mutations, mostly missense, have so far been described in association with PKD, with 529A and 1456T being considered to be the most common mutations in Caucasians.[4,5] R510Q, which is the most frequently occurring mutation among Northern European population, has a dramatically decreased stability toward heat thus decreasing the enzyme level in the cell, accounting for the clinically observed PKD in patients who are homozygous for this mutation.[6]

The precise prevalence of PKD is unknown. Literature review has estimated the prevalence of carriers between 1% and 3%, with a prevalence of PKD about 1 per 20,000 in the white population.[7,8] There is a high frequency of PKD among the Pennsylvania Amish community as a result of a founder effect.[9] Clinical features vary widely, ranging from a mildly symptomatic anemia to one that necessitates regular transfusions. Since the hemolytic process is chronic and begins since birth kernicterus is the commonest symptom, but the frequency of complications such as jaundice, gallstones, iron overload, severe anemia, thrombosis, and osteopenia is well documented.[10–12]

2 | CASE-REPORT

AS, 15-year-old male of Omani origin, presented at the age of 3 years with transfusion-dependent anemia (12 transfusions/year) along with recently initiated oral iron chelation therapy, for iron overload. Family history was not relevant for both parents and siblings. On examination, the patient has pallor, icterus, with a dry skin (diagnosed as Ichthyosis vulgaris). His vitals were normal (BP = 115/75 mm Hg, PR = 75/min, RR = 16/min, Temp = 36.5^{0}C), but per abdomen he had a 7 cm soft, non-tender splenomegaly with 4cms hepatomegaly below the costal margins. Investigations revealed Hb of 8.7 g/dl, MCV 75 fL, reticulocytes count 4.7%, haptoglobin less than 0.06g/L, ferritin 1628 ug/L, bilirubin 18–45 umol/L, and LDH 543 U/L. Hemoglobin variant analysis was normal (HbA 96.3, A2 3.1, and F 0.6). Measuring of enzyme levels initially was difficult due to repeated blood transfusion. Blood smear showed with dimorphic picture, mild polychromasia, and numerous NRBCs (Table 1). Bone marrow examination showed erythroid hyperplasia, dyserythropoiesis with megaloblastic features, binuclear or multinuclear nucleated red cells, and cytoplasmic bridging (Figure 1a & b). He was thus diagnosed as a case of congenital dyserythropoietic anemia (CDA) and investigated with several repeated bone marrow examinations with no different outcomes. Family history revealed that both the parents were consanguineous.

DNA studies were therefore performed by mutational screening for CDA I causative genes (*CDAN1* and *C15ORF41*) by Sanger sequencing. Two rare variants were identified: namely c.256C>T, p. Pro86Ser (rs543791953, MAF <0.01), inherited from the mother, and c.773+107G>T, inherited from the father. Despite both variants being found in *trans* in the proband, subsequent analysis showed that the same genotype was identified in an unaffected brother of the proband, suggesting that this genotype was not the causative of the condition in the proband. Hence, the diagnosis of CDA was ruled out.

C15ORF41 mutational analysis showed the presence of several single nucleotide variants with unknown significance namely rs3743337, rs11073191, rs3784678, rs10220785, and rs6495863. However, no causative mutations in *C15ORF41* gene were found.

Thus, DNA of the proband and both parents was further analyzed by means of targeted next-generation sequencing (t-NGS) approach using a custom panel of 71 genes involved in the RBC disorders by previously described.[13] This panel included causative and candidate genes involved in RBC membrane defects, RBC enzymatic defects, CDA, and Diamond-Blackfan anemia.

TABLE 1 Hematological and Biochemical parameters in the PKD kindred

	Initials, Genotype c.898G>C; p. Ala300Pro	Relation	Sex, Age (years)	Hb(g/dl) RR 11.5–14.5	Retic RR 0.2–2(%)	Total Bilirubin (umol/L) RR 0–17	Haptoglobin RR 0.36–1.95 (g/L)
I.1	SH, Heterozygous	Father	M,45	13.3	1.6	4	1.2
I.2	FA, Heterozygous	Mother	F,38	12.6	1.8	3	1.3
II.1	AS, Heterozygous	Sister	F,21	11.6	1.6	5	0.9
II.2	OS, Heterozygous	Brother	M,19	12.3	1.7	2	1.1
II.3	MS, Heterozygous	Sister	F,17	11.8	0.8	3	1.2
II.4	SS, Normal	Sister	F,16	11.7	1.1	4	1.1
II.5	AS, Homozygous	Proband	M,15	8.7	4.7	18–45	<0.06
II.6	SS, Heterozygous	Sister	S,12	12.2	1.3	2	1.2

The filtering of gene variants was carried out by means of a dedicated pipeline and in agreement with the guidelines of American College of Medical Genetics and Genomics (ACMG).[13] The data obtained by t-NGS were confirmed on the DNA of the patient and his relatives by Sanger sequencing. It was demonstrated that the patient was homozygous for the nucleotide replacement NM_000298.6: c.898G>C that leads to the amino acidic substitution, p. Ala300Pro, whereas both his parents were heterozygous for the same mutation (Figure 2). The other unaffected siblings did not show homozygosity for this variant.

An EDTA blood sample was collected from the proband, 90 days after the last transfusion, subsequently the buffy coat was removed and the RBCs PK activity assay was performed by standard spectrophotometry method.[14] The results compared with enzyme activity obtained from control samples with the same degree of reticulocytosis. Moreover, hexokinase (HK) activity was simultaneously estimated as another cell age-dependent enzyme. Proband PK activity was 4.5 U/g Hb (reference range, 5.6–14 U/g Hb), and HK activity was 1.5 U/g Hb (reference range, 0.7–1.7 U/g Hb).

3 | DISCUSSION

Clinical diagnosis of hereditary hemolytic anemias is difficult and often inaccurate due to overlapping of phenotypes especially in some countries like Sultanate of Oman with high prevalence of hereditary hemolytic anemia and high consanguinity. CDAs are the most difficult to diagnose, if we consider that dyserythropoiesis appears to be a common morphological feature in several conditions and it may be mistaken for Myelodysplastic Syndrome (MDS).[15] With no doubt, the NGS-based testing has revolutionized and improved both diagnosis and management of patients with hereditary anemias.[16]

Our patient had a long-standing history of chronic anemia, jaundice, and splenomegaly along with laboratory studies suggesting an underlying hemolytic anemia. The red cell morphology on the blood film was rather unremarkable and did not show any evidence of a membranopathy implying a diagnosis of chronic nonspherocytic hemolytic anemia (CNSHA). Biochemical studies showed increased LDH, total bilirubin with reduced serum haptoglobin but a negative coombs test. Moreover, the patient needed periodic support with RBC transfusions. The bone

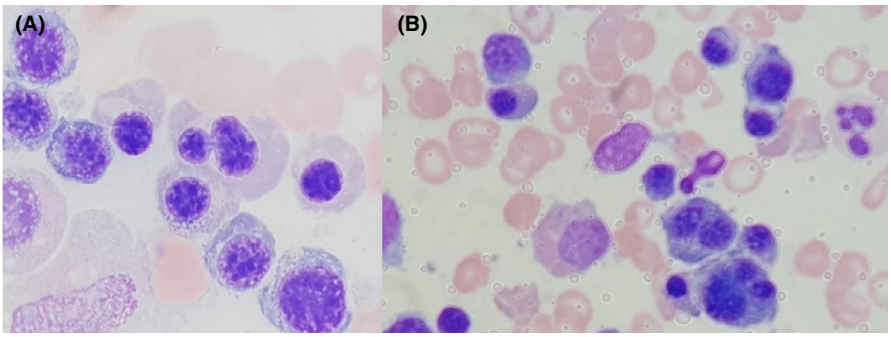

FIGURE 1 Bone marrow showing megaloblastic features, binuclear or multinuclear nucleated red cells and cytoplasmic bridging

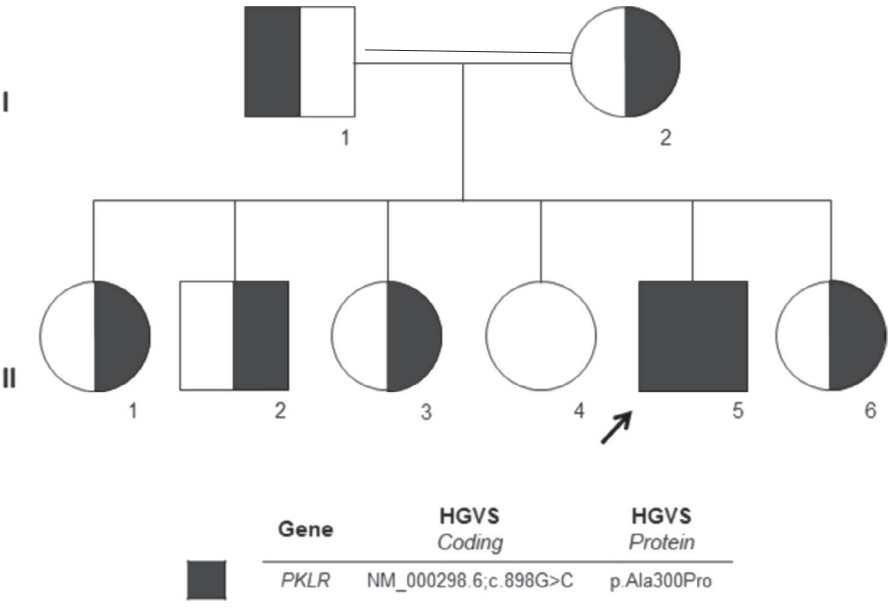

FIGURE 2 Pedigree analysis of the inheritance pattern of the PKD kindred

morrow examination did not reveal any diagnostic pointers but showed dyserythropoiesis with subtle megaloblastic features, cytoplasmic bridging and nuclear irregularity. Thus, a working diagnosis of congenital dyserythropoietic anemia (CDA) was made, and the patient was followed with blood transfusions 6–8 weeks with oral iron chelation therapy.

CDAs are a group of rare inherited RBC disorders characterized by mutations affecting RBC development in the bone marrow. Like PKD, CDA cause chronic hemolytic anemia, jaundice and splenomegaly, and, later in life, iron overload may develop. Moreover, similar to PKD, the RBC morphology may be normal or may show nonspecific abnormalities, but, unlike PKD, in the CDAs, the reticulocyte count is low and the bone marrow shows various abnormalities in developing RBC precursor cells.[15]

Clinically, inherited anemias, that is, disorders due to hemoglobin mutations such as sickle cell disease, unstable hemoglobins or hemoglobin H disease, or RBC membrane defects such as hereditary spherocytosis or hereditary elliptocytosis could have resulted in the same clinical manifestations of our patients but relevant investigations ruled the above.

Lastly, acquired hemolysis or hemolytic anemia due to both intrinsic/intracorpuscular and extrinsic/extracorpuscular RBC defects would also explain the clinical features. Again like PKD, the age of presentation and severity of anemia are variable, the reticulocyte count is increased, and the peripheral blood smear may be unrevealing although occasionally spherocytes may be present, but unlike PKD, in acquired hemolysis and hemolytic anemia, a causative condition or medication can usually be identified from the medical history, medication list, or laboratory testing such as Coombs testing or EMA (eosin-5′-maleimide) flow cytometry, which may reveal antibodies to RBCs or other defects such as absence of glycosylphosphatidylinositol (GPI)-anchored proteins.

PKD is the most frequent enzyme abnormality of the Embden-Meyerhof pathway causing hereditary CNSHA.[17] PKD is an autosomal recessive disorder; with affected individuals being either homozygous for a single pathogenic mutation or compound heterozygous for two different pathogenic mutations affecting the function of the PK enzyme in RBCs and liver[5,12] Individuals who are heterozygous for PK deficiency have intermediate enzyme levels and are not affected clinically. The *PKLR* gene is located on chromosome 1q21. The *PKLR* gene encodes the L (liver) and R (RBC) isoenzymes. The R isoform, unique to RBCs, is 33 amino acids larger than the L isoform, which is unique to hepatocytes. Expression in RBCs versus liver is due to differential use of tissue-specific promoters, which drive expression and tissue-specific exon usage (use of exon 1 but not exon 2 in RBCs and exon 2 but not exon 1 in liver) with over 350 pathogenic variants having been reported on the *PKLR* gene.[5,12,18]

In a study on 254 patients with molecularly confirmed PKD, perinatal complications including anemia that required transfusions, hyperbilirubinemia, hydrops, and prematurity were common.[19] Further, 93% newborns were treated with phototherapy, and almost half were treated with exchange transfusions (46%). Almost 3/4th had to undergo splenectomy resulting in a significant reduction in the need for blood transfusion by 90%. Almost half of the patients in this cohort had iron overload (48%) and gallstones (45%), but other complications such as aplastic crises, osteopenia/bone fragility, extramedullary hematopoiesis, postsplenectomy sepsis, pulmonary hypertension, and leg ulcers were rare. Further, in those who had a splenectomy without simultaneous cholecystectomy, 48% later required a cholecystectomy. It is thus recommended that diagnostic testing for PKD should be considered in patients with apparent congenital hemolytic anemia and close monitoring for iron overload, gallstones, and other complications is needed regardless of baseline hemoglobin.[19]

Causative mutations in homozygous state or compound heterozygous state in this gene have been already described as causative of PKD. Further, it has been recently demonstrated that several patients presenting with clinical data and morphological features of the bone marrow mostly were resembling those of both CDAI and CDAII.[13] The variant identified in the proband is neither currently annotated in public databases nor described as causative mutation of PKD,[5] but following the guidelines by ACMG/AMP 2015 for the clinical interpretation of genetic variants, this novel variant is classified as pathogenic.[20] Whereas PK activity assay for the proband showed lower level compared to matched control, HK activity was within normal. Thus the diagnosis is highly in favor of private kinase deficiency.[21] The findings a also harmonize with the molecular heterogeneity of pyruvate kinase deficiency.[5]

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

Naglaa Fawaz, Alauldeen Alqasim, and Anil Pathare contributed to hematological diagnosis. Ismail Beshlawi and

Mathew Zachariah provided clinical care for the patient. Roberta Russo, Immacolata Andolfo, Antonella Gambale, and Achille Iolascon provided molecular diagnosis. Naglaa Fawaz and Ismail Beshlawi contributed to conception, literature review, and drafting. Alauldeen Alqasim contributed to drafting, manuscript preparation, and revision. All authors read and approved the final manuscript.

ETHICAL APPROVAL

Approval of the institutional ethical committee was waived since this a case report.

CONSENT

A written informed consent was obtained from the patient's guardian prior to submission.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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