Title: Pyruvate Kinase Deficiency in Children

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Abbreviation Key

PKD	Pyruvate Kinase Deficiency
PK	Pyruvate Kinase
RBC	Red blood cells
PKD NHS	Pyruvate Kinase Deficiency Natural History Study
LIC	Liver iron concentration
DW	Dry weight
LDH	Lactate dehydrogenase
ARC	Absolute reticulocyte count
MRI	Magnetic resonance imaging

Abstract

Background. Pyruvate Kinase deficiency (PKD) is a rare, autosomal recessive red blood cell enzyme disorder which leads to lifelong hemolytic anemia and associated complications from the disease and its management. **Methods.** An international, multicenter registry enrolled 124 individuals younger than 18 years old with molecularly confirmed PKD from 29 centers. Retrospective and prospective clinical data were collected. **Results.** There was a wide range in the age at diagnosis from 0-16 years. Presentation in the newborn period ranged from asymptomatic to neonatal jaundice to fulminant presentations of fetal distress, myocardial

depression, and/or liver failure. Children <5 years old were significantly more likely to be transfused than children >12-<18 years (53% vs. 14%, p=0.0006), which correlated with the timing of splenectomy. Regular transfusions were most common in children with two severe *PKLR* variants. In regularly transfused children, the nadir hemoglobin goal varied considerably. Impact on quality of life was a common reason for treatment with regular blood transfusions and splenectomy. Splenectomy increased the hemoglobin and decreased transfusion burden in most children but was associated with infection or sepsis (12%) and thrombosis (1.3%) even during childhood. Complication rates were high, including iron overload (48%), perinatal complications (31%), and gallstones (20%). **Conclusions.** There is a high burden of disease in children with PKD with wide practice variation in monitoring and treatment. Clinicians must recognize the spectrum of the manifestations of PKD for early diagnostic testing, close monitoring, and management to avoid serious complications in childhood.

Introduction

Pyruvate Kinase deficiency (PKD) is the second most common red blood cell (RBC) enzyme disorder causing hereditary hemolytic anemia after glucose-6-phosphate dehydrogenase (G6PD) deficiency.[1] This enzymatic defect in the glycolysis pathway is inherited in an autosomal recessive manner, resulting from compound heterozygous or homozygous mutations in the *PKLR* gene located on chromosome 1 (1q21). Erythrocytes lacking pyruvate kinase (PK) are unable to catalyze phosphoenolpyruvate to pyruvate, resulting in reduced adenosine triphosphate generation that leads to reduced reticulocyte and RBC survival by altering rheology, forming echinocytes that are susceptible to hemolysis, and increased splenic uptake.[2,3] PK deficient RBCs have increased accumulation of 2,3-bisphosphoglycerate, which

shifts the oxygen dissociation curve to the right and decreases the affinity of hemoglobin for oxygen.[4,5]

While the true prevalence of PKD is unknown, population-based studies estimate that PKD occurs in about 51 cases per million in the Caucasian population.[6] The discrepancy between this estimate and the number of patients in clinical practice may be because of its increased prevalence in certain populations, the founder effect (e.g. Amish community of Pennsylvania), or heterogeneous presentation ranging from hydrops fetalis and fetal demise to mild anemia, the cause of both of which may go undiagnosed.[7,8] Variable clinical findings and complications can hinder the approach to diagnostic evaluation and lead to both under-diagnosis and misdiagnosis of more common congenital hemolytic anemias.[9]

The Pyruvate Kinase Deficiency Natural History Study (PKD NHS) was established in 2013 to better characterize the clinical spectrum, presentation, and current management strategies in this rare anemia.[10] This report will focus on clinical manifestations, management, and complications specific to children and adolescents under 18 years of age.

Methods

Patients

The PKD NHS was opened at 30 centers (United States (n=19), Canada (n=3), Italy (n=1), Czech Republic (n=1), Germany (n=5) and Netherlands (n=1) (Supplemental Table S1). The study was approved by the Institutional Review Board and/or Ethics Committee at each

site. Patients and/or legal guardians provided informed consent and assent where appropriate. Patients were able to participate from afar by signed medical releases or were primarily followed at a center approved to conduct the study. Patients were eligible if they had a genetically confirmed diagnosis of PKD with two identified *PKLR* mutations. If prior genetic testing was not performed on the patient or the results were not available, blood was sent for Sanger sequencing (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Milan or Yale-New Haven Children's Hospital).

At the time of enrollment, patients' medical records were reviewed. All patients <18 years of age at the time of enrollment were included in this data set. Data collected included medical history, physical examination, transfusion history, laboratory, and radiologic studies. Missing medical history was obtained by patient/parent recall. Amish children were analyzed and discussed in parallel through the manuscript, as they are unique in homogeneity of genotype (homozygous splicing variant R479H), management, and laboratory and radiologic testing obtained as part of a site-specific protocol.

Statistical Analysis

Patient demographics, transfusion status, comorbid diagnoses and other disease characteristics were described with frequencies, proportions, medians, means, ranges, and interquartile ranges as appropriate to sample size. A Wilcoxon rank-sum test and Fisher's exact test was used to compare continuous variables between groups or categorical variables, respectively. The Cochran-Armitage trend test was used to compare outcomes (two categories) versus ordinal categories (age groups). Regular transfusions were defined as having ≥6

transfusions over a 12-month period. When actual dates were unknown, an approximate date was reported. Patients were considered to have iron overload if (i) their highest ferritin was >1000 ng/ml, (ii) they received chelation therapy 12 months prior to enrollment, or (iii) their highest liver iron concentration (LIC) was >3 mg/g dry weight liver (DW) on magnetic resonance imaging (MRI) for liver iron quantification (T2*) at any time in their history. Sample sizes are presented for those with available data for each variable. The observational registry data were incomplete for many of the factors analyzed herein. When calculating proportions, the denominator reflects the number of patients with known data, which was often smaller than the particular patient cohort being analyzed. P-values were two-sided, and p-values <0.05 were considered statistically significant.

Results

Study Population and Demographic Characteristics

The PKD NHS enrolled 124 children under 18 years-of-age, including 25 children aged 0-2 years, 27 children aged >2-5 years, 36 children aged >5-12 years, and 36 children aged >12-<18 years (Table 1) from 30 centers. Of these, 96 (77%) patients were from 22 centers in North America and 28 (23%) patients were from 8 centers in Europe. Of the 124 children, 22 (18%) were from the Amish community, with a median age at diagnosis of 1 day (range: 1 day-10 years), while the median age at diagnosis for non-Amish children was significantly older at 0.7 years (range: 0-16.3 years, p<0.0001).

Management

Transfusions in the non-Amish cohort:

Most children (87%, 89/102) received at least one transfusion prior to the age of 18 years. The variability of RBC transfusions per year over the follow-up time points are shown in Table 2. Year-to-year variability was low in non-regularly transfused children with 45/50 (90%) receiving 0 transfusions in years 1 and 2 of the study, and 46/50 (92%) in years 1 and year 3 of the study. Similarly, those who received ≥6 transfusions tended to be consistent from year-to-year with 26/34 (76%) regularly transfused in both year 1 and 2 of the study and 22/33 (67%) in years 1 and 3. Substantial variability was seen in year-to-year in those who received 1-5 transfusions per year.

Regularly transfused: Children < 5 years were significantly more likely to receive regular transfusions than children 12-18 years old (53% (24/45) vs.14% (4/29), p=0.0006), but not compared with children aged 5-12 years old (32% (9/28), p=0.09). The most common reasons for regular transfusions at any time-point before enrollment (n=59) were anemia (53/57, 93%) and patient choice/quality of life (15/46, 33%). In children who received regular transfusions in the 12 months prior to enrollment (n=38), the median transfusion volume per transfusion was 13.6 ml/kg (n=30) at a median interval of every 5.8 weeks (range: 2.7-8.7, n=38) with a median nadir hemoglobin of 7 g/dL (range: 4.3-10.7, n=38). The weight-adjusted volume and interval did not vary by age. The regular transfusion rate varied by *PKLR* genotype: 29.8% (17/57) in patients with missense/missense mutations, 26.9% (7/26) with missense/non-missense mutations, and 68.8% (11/16) with non-missense/non-missense mutations.

Not-regularly transfused: Of the 65 children who were not regularly transfused in the 12 months prior to enrollment, the median number of transfusions per 12-month period was 1 (range: 0-4, n=21) for children <5 years old, 0 (range: 0-5, n=19) for those 5-12 years old, and 0 (range: 0-5, n=25) for those >12-<18 years old. The most commonly reported transfusion triggers were presumed infections (45/87, 52%), stress (6/77, 8%), foods (1/76, 1%), and medications (1/81, 1%).

Transfusion in Infants: Children <1 year-of-age at the time of enrollment (n=10) received a median of 4 (range: 0-13) transfusions per year, including one patient who had never received a transfusion. In four infants who were regularly transfused, the median transfusion volume was 16.1 ml/kg at a median interval of 7.2 weeks (range: 4.0-8.7) with a median nadir hemoglobin of 6.3 g/dl (range: 5.9-7.7).

Transfusions in the Amish cohort:

Most Amish children (95%, 21/22) received at least one transfusion prior to the age of 18 years, and only children ages <5 years received regular transfusions (14% vs. 0% in 5-<18 years old).

Splenectomy

Overall, 42% (52/124) of children underwent splenectomy prior to enrollment: 32% (33/102) in the non-Amish and 86% (19/22) in the Amish cohort. The median age of splenectomy was 4.9 years (range: 0.5-16.4) in the non-Amish cohort and 1.5 years (range: 0.6-

3.2) in the Amish cohort. In the non-Amish cohort, the prevalence of splenectomized patients was only 4% (2/45) in children <5 years old, significantly fewer than in those ages 5-12 years (39%, 11/28, p= 0.0003) or in those ages >12-<18 years (69%, 20/29, p<0.0001). The most common indications for splenectomy were to improve anemia (28/32, 88%), reduce transfusion burden (26/32, 81%), improve quality of life (18/26, 69%), and mitigate jaundice (13/27, 48%). Those who underwent splenectomy due to anemia were older (median age 5 years, range: 1.3-16.4, n=28) compared to reasons other than anemia (median age 1.6 years, range: 0.5-3.9, n=4, p=0.014). Splenectomy increased the baseline hemoglobin in 20/26 (77%) patients by a median of 0.7 g/dl (range: 0.1-3.3) and reduced the transfusion burden in 29/31 (94%) patients.

During the 2 years of follow up on the registry, 14 children (Amish and non-Amish) underwent splenectomy (Fig. 1) to improve anemia (12/14, 86%), reduce transfusion burden (13/14, 93%), improve quality of life (9/14, 64%), and/or reduce jaundice (3/13, 23%). The median number of transfusions in the 12 months prior to splenectomy was 10.5 (range: 0-79, n=14), and, of those with at least one year of follow up, only one remained on regular transfusions.

Among patients who underwent splenectomy during the follow-up period with available data (n=10), the median pre- and post-splenectomy hemoglobin were 7.4 g/dl (range: 5.5-9.3) and 8 g/dl (range: 5.9-9.3), respectively. Laboratory markers that were significantly different between pre and post-splenectomy include a: median absolute reticulocyte count (ARC) of 0.88 x 10^6/μL (range: 0.37-1.95, n=6) vs 8.2 x 10^6/μL (5.37-25, n=6, p=0.03), median percent reticulocyte count of 4.4% (1.1%-11.5%, n=10) vs 33.8% (18%-87.8%, n=10, p=0.002), lactate dehydrogenase (LDH) 1272 U/L (776-1420, n=6) vs 332 U/L (183-783, n=6, p=0.03), total white

blood cell count of 6.6 x 10^9/L (3.2-13.3, n=11) vs 11 x 10^9/L (6.6-27.7, n=11, p=0.005), and platelet count of 237 x 10^9/L (180-492, n=11) vs 683 x 10^9/L (286-1556, n=11, p= 0.001). Indirect bilirubin was not significantly different before and after splenectomy.

Of the 66 splenectomized children (at enrollment and over the 2 years of follow up), 43 (65%) were taking prophylactic antibiotics. Post-splenectomy infections or sepsis occurred in 8 (12%) children; those with infections were older at the time of splenectomy (median age: 5.2 years, range: 3.2-6 years). Four were taking prophylactic anticoagulation at enrollment or over the 2 years of follow up, and two patients (1.3%) developed a deep vein thrombosis prior to the age of 18 years.

Other treatments

The majority of children (at enrollment and over the 2 years of follow up) were taking folic acid (91/123, 74%). Other therapies included antidepressants (3/124, 2%), anxiety medication (3/124, 2%), or ursodiol (2/124, 1%). Ten children (10/124, 8%) were reported to be taking alternative or non-traditional therapies, including: vitamin B12, herbal supplement, acidophilus, chlorophyll, echinacea, garlic, green tea, papaya, and St. Johns wart.

Laboratory Findings

In non-regularly transfused, non-splenectomized children (n=43), the median hemoglobin value was 9.1 g/dl (range: 6.0-12.5 g/dl, n=43), median ARC 0.20 x 10^6/μL (range: 0.13-0.73, n=15), indirect bilirubin 2.8 mg/dl (range: 0.3-19.8, n=29), and LDH 858 U/L (range:

183-3811, n=25, Table 3). In non-regularly transfused splenectomized children, the median hemoglobin value was 8.8 g/dl (range: 4.3-12.8, n=43), median ARC 0.63 x 10^6/μL (range: 0.31-1.17, n=16), indirect bilirubin 2.8 mg/dl (range: 1-6.2, n=20), and LDH 213 U/L (range: 154-504, n=13).

Complications

Prenatal complications

Of the enrolled children, 38/122 (31%) had prenatal or neonatal complications, including pre-term birth (15/37, 41%), hydrops fetalis (8/38, 21%), intrauterine growth retardation (5/38, 13%), fetal distress (4/38, 11%), hepatic failure (1/38, 3%), and myocardial depression (1/38, 3%). Of these patients, a substantial number (13/35, 37%) required perinatal transfusions. In the newborn period, 105/120 (88%) had jaundice; of these, 94/102 (92%) were treated with phototherapy and 33/99 (33%) with exchange transfusion. Other neonatal therapies included ursodiol (n=2), phenobarbital (n=1), and erythropoietin (n=1).

Gallstones

Gallstones occurred in 22% (22/101) of non-Amish and 14% (3/21) of Amish children (Table 1). In the non-Amish cohort, the frequency significantly increased with age with 0% (0/45) diagnosed in children ages 0-5 years, 14% (4/28) of those 5-12 years, and 64% (18/28) of those >12-<18 years ($p<0.0001$). The median indirect bilirubin of non-Amish children with gallstones was 5 mg/dl (range: 1-6.4, n=12) as compared to 2.8 mg/dl (range: 0.3-19.8, n=42) in those who

were not diagnosed with gallstones. Of the non-Amish children, 19% (20/102) underwent cholecystectomy at a median age of 6.7 years (2.6-15.7), with 29% (5/17) of these performed at the time of splenectomy.

Iron overload

Iron overload was diagnosed in 48% (32/66) of non-Amish children. Of the 102 enrolled non-Amish children, 64 had a ferritin measured in the prior 12 months and only 8 had a T2*-based MRI. The diagnosis of iron overload did not appear to vary by age (Fig. 2), occurring in 42% (5/12) of children 0-<2 years, 61% (11/18) 2-5 years, 53% (10/19) >5-12 years, and 35% (6/17) in those >12-<18 years, or by gender (45% (17/38) in males vs 54% (15/28) in females, p=0.6). Of the children with iron overload, 1 in the 0-<2 year-age-group, 2 in 2-5 years, 2 in >5-12 years, and 7 in >12-<18 years were not regularly transfused. The median maximum ferritin was 907 ng/ml (range: 22-13,409) and, on T2* MRI, the maximum liver iron was 5.9 mg/g DW (range: 1.9-20, n=8). In children <18 years of age, there was no correlation between ferritin and LIC (r=0.19, p=0.6, n=7).

In the study population, 28/124 (23%) children received chelation therapy, including 17/88 (19%) <12 years-of-age and 11/36 (31%) 12-<18 years-of-age. The most common chelation regimens were: deferasirox (25/28, 89%), deferoxamine (7/28, 25%), combination therapy (3/28, 11%), and deferiprone (2/28, 7%). None of the

children received therapeutic phlebotomy. Thirty-seven percent (14/38) of the regularly transfused and 7% (6/86) of those not regularly transfused were prescribed one of the above chelation therapies.

Other findings

On physical exam, facial jaundice (23/121, 19%), scleral icterus (53/120, 44%), pallor (45/120, 38%), splenomegaly (30/122, 25%), hepatomegaly (14/122, 11%), dyspnea on exertion (1/118, 1%), and bony expansion (7/118, 6%) were documented.

Discussion

The PKD NHS is an international collaborative effort among 30 hematology centers in North America and Europe, which has increased the understanding of the heterogeneity of the genetics, clinical presentation, complications, and management strategies in this rare anemia. In the absence of evidence-based guidelines, the combination of expert-based guidelines and descriptions of a large pediatric cohorts may provide some guidance to clinicians caring for young patients with PKD (Table 4).[11,12]

Despite advances in the diagnostic evaluation for PKD including improved access to both PK enzyme activity and *PKLR* genetic testing, the age at diagnosis in non-Amish children in this cohort was 0-16 years.[9] The majority of children in this cohort were symptomatic around the time of birth and required RBC transfusions during childhood; thus, the older age at diagnosis may reflect milder disease with variable, sometimes atypical, symptoms which may go unrecognized and result in delayed testing. Delays in diagnosis may also relate to the difficulty

in obtaining accurate diagnostic enzyme assays in transfused patients and/or in accessing genetic testing.[13,14] This report describes the spectrum of manifestations in childhood with the goal of leading to earlier identification, appropriate monitoring and management, genetic counseling, and the opportunity to explore potential novel therapies.

In this cohort, perinatal and neonatal complications were common and varied in severity. Most (92%) of the children had the expected presentation of hemolytic anemia with associated jaundice requiring phototherapy, and just 1 in 9 children diagnosed with PKD did not have jaundice in the newborn period. Some infants had more severe neonatal presentations including hydrops fetalis, fetal distress, hepatic failure, and myocardial infarction, sometimes mimicking metabolic disorders and sepsis.[15,16] Enzyme and/or genetic testing for PK deficiency should be strongly considered in the setting of newborns with such fulminant presentations.

After the newborn period, infants required up to 13 transfusions per year, and overall, 36% required regular transfusions. After infancy, over half of the children <5 years-of-age continue to remain transfusion-dependent, especially in those with two severe *PKLR* mutations. The number of transfusions per year significantly decreased during childhood, which reflects both the timing of splenectomy and the resultant improvement in anemia as well as a reduction in hemolytic triggers from infection with age. In this cohort, while the primary reason to remain on a regular transfusion regimen was anemia, perceived quality of life was a significant factor in the decision-making between providers and patient families along with the obligation to support growth and development in these young children.

As indicated in Table 2, the transfusion requirement from year-to-year was least variable in children who were either regularly transfused or who received no transfusions. In children who received 1-5 transfusions per year, the variability from year-to-year during the study was substantial. This unpredictability likely relates to hemolytic triggers and frequency of intercurrent

illnesses on a year-to-year basis, thus assessing transfusion-dependence, baseline hemoglobin levels, and/or determinations of clinical severity challenging. Furthermore, these data highlight the heterogeneity of PKD with about 13% of non-Amish children never requiring a blood transfusion.

Some of the variability in the transfusion practice may be dictated by the provider-family preference, high levels of 2,3-bisphosphoglycerate in the PK deficient erythrocytes conferring better tolerability to lower hemoglobin, and the practice of splenectomy to improve anemia. This is further reflected in the wide range of pre-transfusion nadir hemoglobin values in those children who are regularly transfused with a median goal nadir of 7 g/dl but with a range from 4.3 to 10.7 g/dl. In the setting of normal growth, development, and absence of clinical evidence of significant ineffective erythropoiesis, the goal hemoglobin is individualized based on a child's symptoms and activity level. Management would be improved by future research to elucidate the effect of transfusion management and individualized hemoglobin nadirs on outcomes.

Forty-two percent of children <18 years-of-age were surgically asplenic before enrollment in the study. The majority of splenectomies were performed in older children for anemia, which may correlate with the increased activity and concentration needed at school with age and, therefore, a desire for a higher baseline hemoglobin level. Quality of life was an important indication for splenectomy in 69% of patients. Splenectomy improved anemia in the majority of children but with only a median hemoglobin increase of 0.7 g/dL in this cohort. Notably, this change in hemoglobin is difficult to interpret and likely an underestimate, since the pre-splenectomy hemoglobin reflects a pre-transfusion nadir rather than a true baseline. In addition to the partial amelioration of the anemia, these patients had an average of ten-fold increase in ARC after splenectomy along with a decrease in LDH reflecting increased survival of the reticulocytes, and mild improvement in hemolysis in some patients post-splenectomy.

Cosmetic concerns due to jaundice are not insignificant in congenital hemolytic anemias, particularly in adolescents in whom differences can lead to bullying and other social impacts.[17] Given the lack of change in bilirubin post-splenectomy, jaundice is not an indication for this procedure, while LDH and reticulocyte survival improved post-splenectomy in the non-regularly transfused group, suggesting that splenectomy may mitigate a severe hemolytic phenotype and reduce transfusion burden in many patients.

The safety of splenectomy was queried in this registry, both for infections and vascular complications. Post-splenectomy infections or sepsis occurred in 12% of those <18 years-of-age, and only half of splenectomized children were prescribed prophylactic antibiotics. Post-splenectomy thrombosis was only reported in 2 patients <18 years-of-age, and its incidence in the adult and pediatric PKD NHS cohort at the time of enrollment was 11%. Splenectomy at a younger age increases the time to acquire vascular complications. A 27-year follow-up study in veterans found two-times increased risk of thrombosis and increased risk for sepsis and related deaths in those with splenectomy.[18,19] The rate of post-splenectomy thrombosis and sepsis in this patient population underline the unmet need for safer treatment approaches in this anemia.[20]

Iron overload seen in patients with PKD is secondary to both chronic hemolysis, ineffective erythropoiesis and transfusion therapy.[10,21] Forty-eight percent of non-Amish children had iron overload across all age ranges. The definition of iron overload for this analysis, which used ferritin and chelation data for only the year prior to enrollment, likely led to an underestimate of the actual prevalence of iron overload in this population. Given that only 64/102 children had ferritin measured in the 12 months prior to enrollment, iron overload is not only under-reported, but its awareness is of high importance to the pediatric community due to its significant impact on growth and organs including liver, heart and bones. In a previous report

from the PKD NHS, there was a significant correlation between ferritin and liver iron concentration (LIC) by MRI ($r=0.45$, $p<0.0001$, $n=45$); however, several individual patients who had relatively low ferritin levels, were found to have iron overload by LIC.[21] Using a ferritin cut off of 1000 ng/ml, the sensitivity to predict LIC >3 mg/kg DW was 53% and the specificity was 100%, whereas, for a ferritin cut off of 500 ng/ml, the sensitivity for LIC >3 mg/kg DW was 90% with a specificity of 67%. Based on this, an MRI is recommended for patients with PKD with a ferritin >500 ng/ml (Table 4). Chelation therapy was prescribed to 7% of children in this cohort who were not regularly transfused, and growth complications were reported in two of these patients. For these reasons, close monitoring for iron overload and growth during chelation is paramount in patients with PKD, irrespective of their transfusion status.

Cholelithiasis was reported in less than a quarter of children, and 20% underwent cholecystectomy before enrollment to the PKD NHS. Of these patients, one third underwent cholecystectomy along with splenectomy. The practice of monitoring for asymptomatic gallstones to prevent complications is variable, and splenectomy does not decrease the risk of gallbladder disease in PKD. Therefore, the rate of gallstones in this cohort may be an underestimate and continued awareness and monitoring are needed.

Given the natural history of PKD in children and the identification of a low rate of screening for several important complications, such as iron overload, the authors have included a consensus management guideline for children with PKD in Table 4. Once PKD is suspected and diagnosed by both low PK enzyme activity (compared to other red cell age dependent enzymes)[14] and genetic testing, subsequent management is largely supportive with transfusions for symptomatic anemia impacting everyday quality of life or growth. Transfusions to maintain an arbitrary hemoglobin value should be avoided. Splenectomy should be considered after 5 years of age in those needing frequent transfusions. It is crucial to counsel families that

response to splenectomy is variable and only partially effective at best and is also associated with a risk for post-splenectomy complications such as thrombosis and infections. Before and after splenectomy, appropriate immunizations should be administered, and patients should be provided with post-splenectomy antibiotics and sepsis guidance. Both transfused and non-transfused patients require regular monitoring (Table 4). We recommend regular monitoring for iron overload using ferritin, and MRI to assess iron in those with ferritin > 500 ug/L irrespective of transfusion status. Long-term management decisions should include consideration of clinical trials of potentially disease-modifying treatments, including PK activators and gene therapy. A phase 2 trial of a PK activator, mitapivat, in adults with PKD demonstrated a hemoglobin increase >1.0 g/dL in 50% of the participants (mean hemoglobin increase of 3.4 g/dl (range: 1.1-5.8 g/dl)) with a relationship between at least one missense *PKLR* variant and likelihood of hemoglobin response.[22] Although hematopoietic stem cell transplant has the potential to cure PKD, current approaches are associated with a relatively high rate of morbidity and mortality compared with standard supportive care.[23,24] Gene therapy may be a future option for transfused patients with severe *PKLR* mutations unresponsive to PK activators.[25-27]

The PKD NHS, like other rare disease observational registries, has limitations and potential biases, including from those undiagnosed or deceased. To decrease recall bias and missing data due to lack of documentation, this analysis includes only the subset of patients who were enrolled when <18 years of age, and does not include data from the childhood of participants enrolled at age ≥18 years. Despite the international nature of the registry, due to the rarity of PKD, a limited number of patients are available for data capture, restricting the ability to make associations. Laboratory data from different centers are grouped together and from patients of different ages and genders, both of which are limitations to the interpretation of

the lab findings. Observational data are only available based on provider practices and routine testing, which is reflected by missing data. Given the medical complexity of patients in this cohort, missing data may lead to biased estimates of the prevalence of symptoms and disease complications.

In this pediatric cohort with PKD, a wide spectrum of clinical manifestations was seen with variability in monitoring, management, and complications. In the newborn period, the widest clinical variation was seen, emphasizing the importance of early recognition and testing for PKD. Significant and severe complications in childhood are under-recognized underlining the need for monitoring in all patients regardless of transfusion status. Regular monitoring and care with a pediatric hematologist are imperative for the management and health maintenance of all affected children.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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LEGENDS

TABLE 1. Clinical characteristics of non-Amish children* with Pyruvate Kinase Deficiency, N=102

Characteristics	**n***	**% or median (range)**
Age at diagnosis (years)	98/102	0.7 (0-16.3)
Gender		
Female	44/102	43%
Male	58/102	57%
Race		
Caucasian	85/102	83%

Black	5/102	5%
Asian	6/102	6%
Other	3/102	3%
Unknown	3/102	3%
Ethnicity		
Hispanic	14/102	14%
Non-Hispanic	83/102	81%
Unknown	5/102	5%
Splenectomy prior to enrollment	33/102	32%
Cholecystectomy prior to enrollment	20/102	20%
Median number of lifetime transfusions**		
Overall	89/102	18 (1-312)
Ages 0-<2 y	19	6 (1-30)
Ages 2-5 y	20	25.5 (3-52)
Ages >5-12y	25	33 (1-149)
Ages >12-<18 y	25	21 (1-312)
Number never transfused		
Overall	13/102	
Ages 0-<2 y	3/22	14%
Ages 2-5 y	3/23	13%
Ages >5-12y	3/28	11%
Ages >12-<18 y	4/29	14%
Regularly transfused?		
<6 transfusions over 12 months	65/102	64%
≥6 transfusions over 12 months	37/102	36%
Complications		
Iron Overload***	32/66	48%
Gallstones[#]	22/101	22%
Extramedullary Hematopoiesis (hepatic)[#]	2/94	2%
Pulmonary Hypertension[#]	3/99	3%

n/a: not applicable

*Sample sizes are those with known data for the given characteristic from the Pyruvate Kinase Deficiency Natural History Study. Those from the Amish community (homozygous R479H mutation) were excluded from this Table due to homogeneity in genotype, management, and screening tests with participation in this study.

Amish children (n=22): Transfusion status: Median lifetime transfusions: 11 (1-153), 5% (1/22) receiving regular transfusions, 5% (1/22) never transfused; 86% (19/22) splenectomized; 14% (3/21) with gallstones; 13% (2/15) with iron overload. * Patients were considered to have iron overload if (i) their highest ferritin was >1000 ng/ml, (ii) they received chelation therapy 12 months prior to enrollment, or (iii) their highest liver iron concentration (LIC) was >3 mg/g dry weight liver (DW) on magnetic resonance imaging (MRI) for liver iron quantification (T2*) at any

time in their history. # The presence or absence of gallstones, pulmonary hypertension, and extramedullary hematopoiesis were reported by sites but patients may not have been screened by imaging.

TABLE 2. Distribution of the number of red cell transfusions per year in children with Pyruvate Kinase Deficiency during a three-year period*

		Year 2*			
	Pediatric patients with known transfusion data in Years 1 and 2 (N=112)	0 transfusions	1-2 transfusions	3-5 transfusions	≥6 transfusions
Year 1*	0 transfusions (n=50)	**45/50 (90%)**	2/50 (4%)	0/50 (0%)	3/50 (6%)
	1-2 transfusions (n=12)	3/12 (25%)	**3/12 (25%)**	4/12 (33%)	2/12 (17%)
	3-5 transfusions (n=16)	3/16 (19%)	4/16 (25%)	**5/16 (31%)**	4/16 (25%)
	≥6 transfusions (n=34)	2/34 (6%)	3/34 (9%)	3/34 (9%)	**26/34 (76%)**
		Year 3*			
	Pediatric patients with known transfusion data in Years 1 and 3 (N=105)	0 transfusions	1-2 transfusions	3-5 transfusions	≥6 transfusions
Year 1*	0 transfusions (n=50)	**46/50 (92%)**	1/50 (2%)	0/50 (0%)	3/50 (6%)
	1-2 transfusions (n=11)	4/11 (36%)	**3/11 (27%)**	2/11 (18%)	2/11 (18%)
	3-5 transfusions (n=11)	3/11 (27%)	3/11 (27%)	**3/11 (27%)**	2/11 (18%)
	≥6 transfusions (n=33)	4/33 (12%)	1/33 (3%)	6/33 (18%)	**22/33 (67%)**

Proportions (percentages) of patients are displayed for the rows

*Year 1: 12-month period before enrollment. Year 2: First year of follow-up after enrollment. Year 3: Second year of follow-up after enrollment.

TABLE 3. Laboratory parameters of children with Pyruvate Kinase Deficiency (n=124)

Laboratory Parameter	**Non-regularly transfused N=86**		**Regularly Transfused N=38**	
	n*	**% or median (range)**	**n***	**% or median (range)**
Genotype				
Missense/Missense	40/64	63%	17/35	49%
Missense/Non-Missense	19/64	30%	7/35	20%
Non-Missense/Non-Missense	5/64	8%	11/35	31%
Hemoglobin (g/dL)				
Non-splenectomized	43	9.1 (6-12.5)	29	7.6 (4.3-10.7)
Splenectomized	43	8.8 (4.3-12.8)	9	9.3 (7-9.8)
Absolute Reticulocyte Count (x 10^6/uL)				
Non-splenectomized	15	0.20 (0.13-0.73)	11	0.14 (0.07-0.26)
Splenectomized	16	0.63 (0.31-1.17)	2	0.23 (0.16-0.30)
Reticulocyte Percent (%)				
Non-splenectomized	37	6.7 (1.4-82.9)	25	10.35 (0.4-39.1)
Splenectomized	42	24.7 (8.6-61.2)	5	6.05 (2.2-27.7)
Total Bilirubin (mg/dl)				
Non-splenectomized	39	3.4 (0.1-33.1)	22	3.82 (1.3-13)
Splenectomized	34	2.4 (1-6.6)	7	5 (3.3-8.43)
Lactate Dehydrogenase (U/L)				
Non-splenectomized	25	858 (183-3811)	12	926 (347-1987)
Splenectomized	13	213 (154-504)	4	944 (624-1033)
Maximum Ferritin (ng/ml)**				
Non-splenectomized	17	144 (31-13409)	25	979 (22-2988)
Splenectomized	28	552 (170-2786)	9	1440 (423-9679)

*Sample sizes are those with known data for the given characteristic from the Pyruvate Kinase Deficiency Natural History Study. Regularly transfused: ≥6 transfusions, N= 38 (9 splenectomized and 29 non-splenectomized); Not-regularly transfused: <6 transfusions per year, N=86 (43 splenectomized and 43 non- splenectomized).

** Maximum Ferritin was reported in patients independently (prescribed or not prescribed) of chelation treatment.

TABLE 4: Considerations in the management of Pyruvate Kinase Deficiency in children

Confirmatory testing for PK deficiency
1) reduced PK enzyme activity or reduced PK/HK ratio on full RBC enzyme evaluation* and 2) homozygous or compound heterozygous mutations in the *PKLR* gene. *PK deficiency should be suspected in patients of any age with unexplained chronic hemolytic anemia.*
Transfusion management
Indications for Transfusions: • Hemolytic crisis with hyperbilirubinemia in a neonate suspected with PKD** • Symptomatic anemia with impact on everyday quality of life (rather than using an arbitrary hemoglobin value as an indication, suggest transfusions based on symptoms, complications, and/or co-morbidities) • Promote and sustain growth in young children • Severe and/or symptomatic anemia during intercurrent illness or aplastic crisis • Perioperative management
After an initial transfusion, consider extending the duration between transfusions if the patent is relatively asymptomatic and with normal growth.
In the setting of worsening anemia without a clear trigger, investigate for secondary causes such as viral infections, nutritional deficiencies, medication effect, and/or accessory spleen (in splenectomized patients).
Full splenectomy indications
Symptomatic anemia and regular transfusions ***
Monitoring in transfusion and non-transfusion dependent children

	Regularly transfused	Not regularly transfused
CBC, Reticulocytes	Monthly	Every 3-6 months
Height velocity, weight, Pubertal assessment	Every 6 months	Annually
Vitamin D	Annually	Annually
Serum ferritin	Every 6 months	Annually
Magnetic resonance imaging of liver and heart for iron (T2*)	Annually****	Once**** and then subsequent frequency based on findings and ferritin trends
Ultrasound abdomen for gallstones	If symptomatic, and prior to splenectomy	
HIV and viral hepatitis testing*****	Annually	-
Endocrine testing (growth hormone, thyroid function, sex hormones)- starting at 5y of age	Annually	-
DEXA	After puberty, subsequent frequency based on findings	
Imaging for Extramedullary Hematopoiesis (EMH)	If concerns for paravertebral EMH or pain	

* (Al-Samkari et al. 2020). PK- pyruvate kinase, HK- hexokinase DEXA: dual energy x-ray absorptiometry

** Hemolysis in the newborn period can be associated with marked hyperbilirubinemia and risk for kernicterus. If PKD is suspected prior to birth (for example; affected sibling, Amish

background) preparation should be made for monitoring and treatment with phototherapy, simple transfusion(s), and/or exchange transfusion(s) after birth.

*** Recommend additional immunizations before and after splenectomy and lifelong antibiotic prophylaxis and fever guidelines. Recommend deferring until age 5 years, and consider enrolling on clinical trial(s), if available prior to splenectomy, to assess benefit.

**** Recommend first MRI after 10-12 transfusions. In non-transfused patients, consider delaying MRI until ferritin is over 500 ug/L and/or can be performed without sedation.

***** Parvovirus titers should be obtained with concern for an aplastic crisis. Some centers follow parvovirus titers to evaluate the risk of impending infection. Families should be counseled regularly on the signs and symptoms of an aplastic crisis.

Al-Samkari, H., K. Addonizio, B. Glader, D. H. Morton, S. Chonat, A. A. Thompson, K. H. M. Kuo, Y. Ravindranath, H. Wang, J. A. Rothman, J. L. Kwiatkowski, C. Kung, P. A. Kosinski, H. Al-Sayegh, W. B. London, and R. F. Grace. 2020. 'The pyruvate kinase (PK) to hexokinase enzyme activity ratio and erythrocyte PK protein level in the diagnosis and phenotype of PK deficiency', *Br J Haematol*.

FIGURE 1. Hemoglobin level, transfusions, and timing of splenectomy in the follow-up period in children who underwent splenectomy while enrolled in the PKD NHS, N=14

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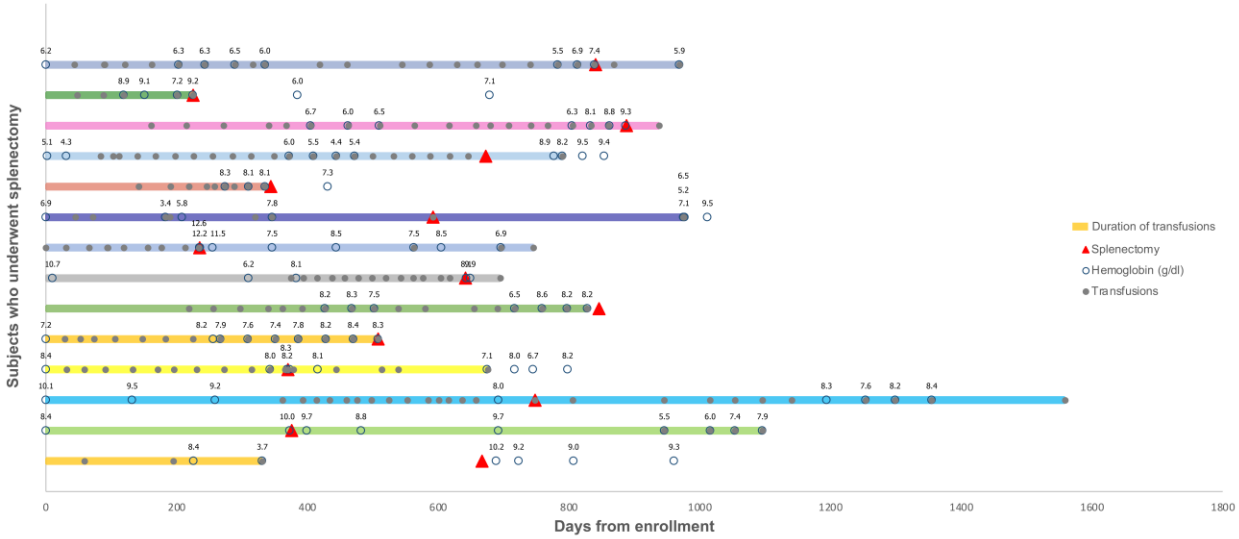

Note: each row represents one child's data.

FIGURE 2. Iron status by age in children enrolled in the PKD NHS for children with known liver iron concentration (LIC) at enrollment, N=22

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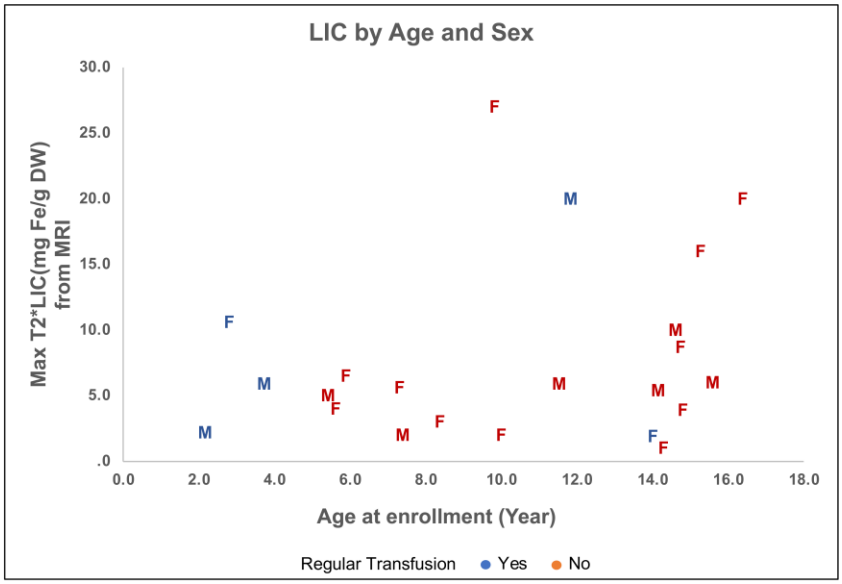

LIC=Liver iron concentration, M=Male, F=Female

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Gender		
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Male	58/102	57%
Race		
Caucasian	85/102	83%
Black	5/102	5%
Asian	6/102	6%
Other	3/102	3%
Unknown	3/102	3%
Ethnicity		
Hispanic	14/102	14%
Non-Hispanic	83/102	81%
Unknown	5/102	5%
Splenectomy prior to enrollment	33/102	32%
Cholecystectomy prior to enrollment	20/102	20%
Median number of lifetime transfusions**		

Overall	89/102	18 (1-312)
Ages 0-<2 y	19	6 (1-30)
Ages 2-5 y	20	25.5 (3-52)
Ages >5-12y	25	33 (1-149)
Ages >12-<18 y	25	21 (1-312)
Number never transfused		
Overall	13/102	
Ages 0-<2 y	3/22	14%
Ages 2-5 y	3/23	13%
Ages >5-12y	3/28	11%
Ages >12-<18 y	4/29	14%
Regularly transfused?		
<6 transfusions over 12 months	65/102	64%
≥6 transfusions over 12 months	37/102	36%
Complications		
Iron Overload***	32/66	48%
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Symptomatic anemia and regular transfusions ***

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