Healthy-Related Quality of Life and Fatigue in Children and Adults with Pyruvate Kinase Deficiency

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Abstract:
Pyruvate kinase deficiency (PKD) is the most common cause of congenital non-spherocytic hemolytic anemia. Although recognition of the disease spectrum has recently expanded, data describing its impact on health-related quality of life (HRQoL) are limited. In this prospective international cohort of 254 patients (131 adults and 123 children) with PKD, we assessed the disease impact on HRQoL (EuroQL-5D, PedsQL, FACT-An) and fatigue (PROMIS Fatigue, PedsFACT-F) using validated measures. Significant variability in HRQoL and fatigue was reported for both adults and children although individual scores were stable over a 2-year interval. While adults who were regularly transfused reported worse HRQoL and fatigue compared to those who were not regularly transfused (EQ-VAS 58 vs. 80, p=0.01), this difference was not seen in children. Regularly transfused adults reported lower physical, emotional, and functional well-being and more anemia symptoms. Both HRQoL and fatigue significantly differed in children by genotype with the worst scores in those with two severe PKLR mutations; this difference was not seen in adults. However, iron chelation was associated with significantly worse HRQoL scores in both children and adults. Pulmonary hypertension was also associated with significantly worse HRQoL. In PKD-specific symptom assessment, 59% of adults and 35% of children reported that their jaundice upset them, identifying this as an important symptom for consideration. While current treatments for PK deficiency are limited to supportive care, new therapies are currently in clinical trials. Understanding the impact of PKD on HRQoL is important to assess the utility of these treatments. (Clinicaltrials.gov number NCT02053480)

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Health-Related Quality of Life and Fatigue in Children and Adults with Pyruvate Kinase Deficiency

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KEY POINTS

- The impact of PK deficiency on HRQoL and fatigue is described in 254 children and adults with the disease using 6 validated instruments.

- Severe anemia, regular transfusion, iron chelation, and non-missense mutations were associated with worse patient-reported outcomes.

ABSTRACT

Pyruvate kinase deficiency (PKD) is the most common cause of congenital non-spherocytic hemolytic anemia. Although recognition of the disease spectrum has recently expanded, data describing its impact on health-related quality of life (HRQoL) are limited. In this prospective international cohort of 254 patients (131 adults and 123 children) with PKD, we assessed the disease impact on HRQoL (EuroQL-5D, PedsQL, FACT-An) and fatigue (PROMIS Fatigue, PedsFACIT-F) using validated measures. Significant variability in HRQoL and fatigue was reported for both adults and children although individual scores were stable over a 2-year interval. While adults who were regularly transfused reported worse HRQoL and fatigue compared to those who were not regularly transfused (EQ-VAS 58 vs. 80, p=0.01), this difference was not seen in children. Regularly transfused adults reported lower physical, emotional, and functional well-being and more anemia symptoms. Both HRQoL and fatigue significantly differed in children by genotype with the worst scores in those with two severe PKLR mutations; this difference was not seen in adults. However, iron chelation was associated with significantly worse HRQoL scores in both children and adults. Pulmonary hypertension was also associated with significantly worse HRQoL. In PKD-specific symptom assessment, 59% of adults and 35% of children reported that their jaundice upset them,
identifying this as an important symptom for consideration. While current treatments for PK deficiency are limited to supportive care, new therapies are currently in clinical trials. Understanding the impact of PKD on HRQoL is important to assess the utility of these treatments. (Clinicaltrials.gov number NCT02053480)
INTRODUCTION

Pyruvate kinase deficiency (PKD) is an autosomal recessive hereditary hemolytic anemia resulting from mutations in the PKLR gene. Though it is the most common cause of chronic hereditary non-spherocytic hemolytic anemia, its precise prevalence remains unclear, with estimates ranging between 1:20,000 and 1:300,000 in Caucasian populations\(^1,2\) and a higher prevalence in malaria endemic areas. Pyruvate kinase (PK) is the rate-limiting step in erythrocyte adenosine triphosphate (ATP) production, and the shortage of ATP resulting from its deficiency results in diminished capacity to maintain the erythrocyte membrane and decreased erythrocyte deformability.\(^3\) This leads to chronic hemolytic anemia due to decreased erythrocyte lifespan and premature splenic erythrocyte destruction. Hemolysis in PKD ranges from mild and asymptomatic to a severe, transfusion-dependent anemia from birth.\(^4\) The sequelae include typical symptoms of anemia (fatigue, reduced exercise tolerance, reduced concentration), iron overload and its complications, extramedullary hematopoiesis, bone disease, endocrinopathies, and venous thromboembolism, among other complications.\(^5,6\)

While data from the international Pyruvate Kinase Deficiency Natural History Study (PKD NHS)\(^5\) and other cohorts\(^7-9\) have been important in defining the disease spectrum, there are little objective or quantitative published data describing the impact of PKD on health-related quality of life (HRQoL) or fatigue. A prior qualitative interview study of 21 adults with PKD described a negative impact of the disease on appearance, emotional and cognitive states, sleep, work and/or school, and the ability to perform physical, social, and leisure activities\(^10\). Studies published in other hereditary hemolytic anemias
such as sickle cell disease\textsuperscript{11} and thalassemia\textsuperscript{12} have demonstrated the importance of defining the impact of chronic hemolytic anemia on patient-reported outcomes, underscoring its significance as an outcome measure in evaluation of treatments. While treatments for PKD are currently limited to red cell transfusions, splenectomy, and the rare hematopoietic stem cell transplant, oral pyruvate kinase activators\textsuperscript{13} and gene therapy\textsuperscript{14} are currently in clinical trials. Understanding the impact of PKD on HRQoL and fatigue is critical to fully characterize the value and utility of these treatments. Therefore, this study aimed to characterize the disease impact on HRQoL and fatigue in an international population of children and adults with PKD.

**METHODS**

*Patient Population*

The PKD NHS (NCT02053480) was opened at 30 centers (United States (n=19), Canada (n=3), Italy (n=1), Czech Republic (n=1), Germany (n=5), Netherlands (n=1), Supplemental Table 1). The study protocol was performed in accordance with the Declaration of Helsinki, approved by the Institutional Review Board and/or Ethics Committee at each site, and all patients and/or their legal guardians gave informed consent. 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 to be included in the registry study if they had a genetically confirmed diagnosis of PKD with two identified \textit{PKLR} mutations. At the time of enrollment, patients' medical records were reviewed retrospectively, and patients were then followed prospectively for 2 years. Data collected from the medical record included medical history, physical examination,
and laboratory and radiologic studies. Medical history missing from the medical records was obtained by patient recall, if known. HRQoL was measured at three timepoints, at enrollment and at the 1 and 2-year follow-up timepoints in both children (<18 years) and adults (≥18 years).

**Patient-Reported Outcome Measures**

**Adult Measures**

**Measurement of HRQoL:** In participants ≥18 years, the EuroQol 5-Dimension Questionnaire (EQ-5D-5L) was utilized (Supplemental Table 2). The EQ-5D is a generic HRQoL instrument that includes two primary components: a descriptive system used to generate a health utility score and the EuroQol-visual analog scale (EQ-VAS), in which respondents mark health status on a vertical scale with end points of 0 (worst health) and 100 (best health). EQ-5D scoring has been validated across multiple chronic diseases and in different countries. The Functional Assessment of Cancer Therapy-Anemia (FACT-An), a validated survey which measures general HRQoL concerns (physical, social, emotional, and functional well-being) plus items specifically related to anemia and fatigue, was utilized for measurement of adult HRQoL. Scores range from 0-188, in which a higher score indicates a higher level of HRQoL (Supplemental Table 2).

**Measurement of Fatigue:** The Patient Reported Outcomes Measurement Information System (PROMIS) Fatigue Short Form was utilized to measure fatigue in adults aged ≥18 years (Adult self-report 7a). PROMIS raw scores are converted to T-scores, in which a standardized score has a mean of 50 and a standard deviation of 10. Higher
scores indicate more fatigue. English-speaking adults were asked an additional question for symptom assessment specific to PKD (“I got upset about my jaundice (yellow eyes/skin)”) with a 0-5 ranking scale (0=not at all, 5=very much).

**Pediatric Measures**

*Measurement of HRQoL:* In children and adolescents, the Pediatric Quality of Life Inventory Generic Core Scale version 4.0 (PedsQL), a validated tool which measures across the dimensions of physical, emotional, social, and school functioning, was completed (**Supplemental Table 2**). Parent proxy forms were completed for children aged 2-17 years and patient self-report forms were completed for children aged 5-17 years. PedsQL scores have a possible range of 0 (worst) to 100 (best).

*Measurement of Fatigue:* The Pediatric Functional Assessment of Chronic Illness Therapy-Fatigue (Peds-FACIT-F) was used to measure fatigue reported by children aged 8-17 years. Scores are measured from 0-52, in which a lower score indicates a higher level of fatigue. The PROMIS Fatigue Short Form was utilized to measure fatigue in children aged 8-17 years (child self-report 10a) and parent proxy (parent proxy 10a) in children aged 5-17 years. Analysis of the child and parent proxy short forms is the same as the adult forms, in which higher scores indicate more fatigue. English-speaking children were asked an additional question for symptom assessment specific to PKD (“I got upset about my jaundice (yellow eyes/skin)”) with a 0-5 ranking scale (0=none of the time, 5=all of the time).

**Definitions and Statistical Analysis**
Patient demographics, transfusion status, comorbid diagnoses and other disease characteristics were described with frequencies, proportions, medians, means, and ranges. Patients were considered regularly transfused if they had received ≥6 red cell transfusion episodes in the prior year. Iron overload was defined either by elevated ferritin (ferritin ≥1000 ng/mL) or by use of iron chelation at any point. Since the Amish population represented a large and similarly managed subset of the cohort, analyses were performed for both the entire cohort and for Amish population separately. Tests of association were performed using the Fisher’s exact test for categorical data, and the Wilcoxon rank-sum test or the Kruskal-Wallis test for continuous data. Sample sizes are presented for those with known data available for each variable. Correlations reported are Spearman. Data were collected using Inform and analyzed with SAS v9.4 (Cary, NC). Surveys were unavailable in specific languages which led to more missing data for certain measures (Supplemental Table 2). The EQ-5D summary health utility score is computed using country-specific value sets. P-values are two-sided, and p-values <0.05 were considered statistically significant.

RESULTS

PKD NHS Demographics

Patients with PKD were enrolled from June 2014 through April 2017 at 30 centers in 6 countries. A total of 254 eligible participants are reported herein including 131 adults (≥18 years of age) and 123 adolescents and children (<18 years of age). There were 55 patients (21.7%) who identified as part of the Amish community. Baseline demographic information is presented in Table 1. A comprehensive characterization of the
complications and laboratory abnormalities associated with PKD from this population are described in a separate publication.\textsuperscript{5} Completed HRQoL surveys varied by age and measure (Table 2, Supplemental Table 2).

\textit{Report of Overall HRQoL of Life in Adults with PKD}

\textit{EQ-5D-5L.} The EQ-5D Visual Analog Scale was completed by 120 of the 131 adults at enrollment, 98 at year 1 and 88 at year 2. The median EQ-VAS score was 80 with significant variability in the range of scores reported (20-100). The median EQ-health index score was 0.88 with a range from 0.43-1. The median EQ-VAS and health index score were unchanged at years 1 and 2 of follow-up.

At the time of enrollment, patients aged $\geq 40$ years reported significantly lower EQ-VAS scores compared with adults aged 18-39 years (80 vs. 85, p=0.02). Scores were lower in women vs. men (80 vs. 85, p=0.03), non-Amish vs. Amish (80 vs. 85, p=0.04), regularly-transfused vs. not regularly transfused (58 vs. 80, p=0.01), iron overload by chelation requirement vs. not (75 vs. 84, p=0.02), and with pulmonary hypertension vs. without (40 vs. 80, p=0.03). When the Amish population was excluded from the analysis, there were no longer significant differences by age or history of iron overload by chelation requirement. EQ-VAS scores did not differ based on splenectomy status (p=0.67), hemoglobin ($<8$ g/dl vs. $>8$ g/dl, p=0.97), bilirubin level (total bilirubin $>4$ mg/dl vs $<4$ mg/dl, p=0.97), number of lifetime transfusions (p=0.20), iron overload by ferritin (p=0.12), or history of extramedullary hematopoiesis (p=0.31). EQ-VAS scores also did not differ by genotype category: missense/missense (median 75),
missense/non-missense (median 83), or non-missense/non-missense (median 80, p=0.28).

**FACT-An.** The FACT-An was completed by 126 adults at enrollment, 98 at year 1 and 88 at year 2. The median FACT-An score was 155.6 with high variability between individual patient scores, which ranged from 33.3 to 181. The median FACT-An score was similar at years 1 and 2 of follow-up.

At enrollment, the report of HRQoL using the FACT-An did not vary by age. Significantly lower HRQoL were reported by women vs. men (median 143 vs. 159, p=0.008), non-Amish vs. Amish (151 vs. 163, p=0.04), regularly transfused vs. not regularly transfused (113 vs. 157, p=0.0003), iron overload by chelation requirement vs. not (146 vs. 157, p=0.006), and with pulmonary hypertension vs. without (99 vs. 157, p=0.002). Report of HRQoL did not differ based on splenectomy status (p=0.95), hemoglobin (<8 g/dl vs >8 g/dl, p=0.97), bilirubin level (total bilirubin >4 mg/dl vs <4 mg/dl, p=0.4), number of lifetime transfusions (p=0.18), or iron overload by ferritin (p=0.12). There were no significant differences based on PKLR genotype (p=0.63). Differences, or the lack thereof, in FACT-An scores in certain subgroups are illustrated in Figure 1.

**FACT-An Subscales.** Within the FACT-An, differences were noted in specific subscales across clinical characteristics. Lower scores in the social well-being subscale were reported by patients ≥40 years vs. <40 years (22 vs. 26, p=0.0006) and those who had not been splenectomized vs. those post-splenectomy (21 vs 25, p=0.004). Additionally,
lower scores in the anemia subscale were reported for females vs. males (58 vs. 67, p=0.001). Regularly-transfused adults reported significantly lower physical well-being (p=0.002), emotional well-being (p=0.004), and functional well-being (p=0.0007) as well as increased anemia symptoms (p=0.0002). Those with a history of iron overload by chelation requirement also reported lower physical well-being (p=0.0001), emotional well-being (p=0.001), and functional well-being (p=0.0007) as well as increased anemia symptoms (p=0.02). In addition, pulmonary hypertension was associated with significantly lower physical well-being (p=0.0004) and functional well-being (p=0.005) as well as increased anemia symptoms (p=0.0008).

Report of Fatigue in Adults with PKD

PROMIS-SF7a. The PROMIS SF-7a was completed by 60 adults at enrollment and by 47 and 36 adults at years 1 and 2, respectively. The lower number of respondents for this inventory reflects the lack of validated translated forms for some of the participating countries. The median PROMIS SF-7a score was 52.3 (range: 29.4-80.3), and this median was replicated at both years 1 and 2.

In adults, the report of fatigue using the PROMIS SF-7a did not vary by age or by Amish or non-Amish status. Significantly higher levels of fatigue were reported by women vs. men (57 vs 51, p=0.004), regularly transfused vs. not regularly transfused (68 vs. 52, p=0.03), and with pulmonary hypertension vs. without (65 vs. 52, p=0.04). Report of fatigue did not differ based on splenectomy status (p=0.59), hemoglobin (<8 g/dl vs >8 g/dl, p=0.93), bilirubin level (total bilirubin >4 mg/dl vs <4 mg/dl, p=0.28), number of lifetime transfusions (p=0.37), history of extramedullary hematopoiesis
(p=0.43), iron overload by ferritin (p=0.12), or iron overload by chelation (p=0.20). Report of fatigue did not differ by PKLR genotype category (p=0.79).

Other report of symptoms of anemia. English-speaking adults were asked an additional question for symptom assessment specific to PKD (Table 3). Of the 68 adults who completed the questionnaire, 59% (40/68) reported that their jaundice upset them and 16% (11/68) reported that they felt quite a bit or very much upset.

Report of Overall HRQoL in Children with PKD

The PedsQL 4.0 survey was completed by 61 of the 124 children at enrollment, 45 at year 1 and 48 at year 2. The parent proxy was completed by 86 of 124 parents at enrollment, 72 at year 1, and 59 at year 2. The median child self-report score was 82.6 (range: 33.7-100), and the median parent-proxy score was 83.5 (range: 25-100). The median child self-report and parent proxy score were unchanged at years 1 and 2 of follow-up.

The report of HRQoL did not differ by age group in children (ages 2-4, 5-7, 8-12, vs. 13-17 y, p=0.34) or by their gender (p=0.69). Amish children generally reported higher HRQoL scores (93 vs. 80, p=0.007). HRQoL was significantly lower in children who were more anemic (<8 g/dl vs. >8 g/dl, 69 vs. 91, p=0.01), had iron overload by ferritin (34 vs. 92, p=0.006), and had iron overload by chelation requirement (70 vs. 88, p=0.007). HRQoL scores also significantly differed by PKLR genotype with those with a non-missense/non-missense genotype reporting a significantly lower HRQoL (missense/missense 83 vs. missense/non-missense 84 vs. non-missense/non-missense
The report of HRQoL did not differ based on splenectomy status (p=0.64), bilirubin level (total bilirubin >4 mg/dl vs. <4 mg/dl, o=0.69), receipt of regular transfusions or not (p=0.21), total number of lifetime transfusions (p=0.06), iron status by ferritin (p=0.12), or history of extramedullary hematopoiesis (p=0.07). When Amish children were excluded from the analysis, significantly lower HRQoL was reported in children who were splenectomized (76 vs. 83, p=0.02) and in those receiving a higher number of lifetime transfusions (77 vs. 82, p=0.02). However, there was no longer a significant difference in HRQoL by hemoglobin level (p=0.23).

Report of Fatigue in Children with PKD

Peds-FACIT-F. The Peds-FACIT-F was completed by 47 older children and adolescents at enrollment and 40 and 36 at years 1 and 2, respectively. The median score at enrollment was 45 (range, 16-52) and was unchanged at years 1 and 2. In this cohort at enrollment, there was no difference in report of fatigue by age group (p=0.17), gender (p=0.46), splenectomy status (p=0.76) number of lifetime transfusions (p=0.14), current transfusion status (p=0.09), hemoglobin (p=0.41), bilirubin level (p=0.54), or iron overload by ferritin (p=0.83). Amish children reported fatigue similar to that of non-Amish children (median: 46 vs. 45, respectively, p=0.14). The report of fatigue significantly varied by PKLR genotype with those with a non-missense/non-missense genotype reporting higher levels of fatigue (missense/missense 46 vs. missense/non-missense 45 vs. non-missense/non-missense 34, p=0.047). Children with a history of chelation therapy also reported higher levels of fatigue (median: 40 vs 46, p=0.03).
Differences, or the lack thereof, in Peds-FACIT-F scores in certain subgroups are illustrated in Figure 2.

**PROMIS Child SF-10a.** The PROMIS Child SF-10a was completed by 36 older children and adolescents with a median score of 41.6 (range: 30.3-65.7). The PROMIS Parent Proxy was completed by 48 parents with a median score of 46.3 (range: 34.1-69.2). The median child self-report and parent proxy report were unchanged at follow-up. There was no significant difference in the report of fatigue by age (p=0.31), gender (p=0.82), splenectomy status (p=0.88), number of lifetime transfusions (p=0.07), transfusion status (p=0.06), hemoglobin (p=0.18), bilirubin level (p=0.94), iron overload by ferritin (p=0.12), or iron overload by chelation requirement (p=0.19). There was also no significant difference in report of fatigue by *PKLR* genotype (median scores for missense/missense 42, missense/non-missense 44, non-missense/non-missense 56, p=0.08). Although there was no difference in report of fatigue between Amish and non-Amish children as measured by PROMIS, when Amish children were excluded from analysis, there was a significantly higher level of fatigue reported in children with a history of iron overload by ferritin (57 vs. 32, p=0.008) and by chelation requirement (57 vs. 41, p=0.009).

**Other report of symptoms of anemia.** English-speaking children were asked additional questions generated for symptom assessment specific to PKD (Table 3). Of the 40 children who completed the questionnaire, 35% (14/40) reported that their jaundice
upset them at least some of the time, and 7.5% (3/40) reported that they felt upset most or all of the time.

DISCUSSION

This study is the first report of HRQoL and fatigue in patients with PKD using validated instruments. A wide range of scores were reported for general HRQoL as measured by the EQ-5D in adults and the PedsQL 4.0 in children and for fatigue using the FACIT-F in adults, Peds-FACIT-F in children, and PROMIS in all ages. Consistent correlates were identified between specific clinical characteristics in patients with PKD and worse overall HRQoL and fatigue (Table 4).

The median reported general HRQoL as measured by the EQ-5D in adults and the PedsQL 4.0 in children approximated that of the general population. There are numerous possible explanations for this finding. There is a very wide range of disease spectrum in the analyzed cohort, from asymptomatic individuals with no anemia to individuals transfusion-dependent since birth. Consistent with the high variation in symptoms and complications, some patients reported very low HRQoL or high level of fatigue whereas others reported the best possible HRQoL or no fatigue. In addition, adjustment to chronic anemia and a lack of recognition of its impact on functional status is well-described in populations with congenital anemia, and this may also be responsible for the observed findings. Another possibility lies with the selected instruments, which, while validated tools, are not validated for use in PKD, hemolytic anemias, or congenital anemias. Therefore, they may be more sensitive to acquired anemia, such as the anemia associated with chronic kidney disease or cancer. The
instruments may be insensitive to aspects of lifelong hemolytic anemia that reduce HRQoL, including iron overload, chronic jaundice, and pulmonary hypertension. Finally, there may be specific aspects of PKD that modulate the impact of chronic anemia, such as the elevation in 2,3-diphosphoglycerate (2,3-DPG). 2,3-DPG is an important regulator of the oxygen affinity of hemoglobin and is increased in PKD due to the metabolic block of glycolysis resulting in an upstream accumulation of glycolytic intermediates. Therefore, enhanced oxygen delivery due to elevated 2,3-DPG may obviate symptoms of anemia in some patients.

While these factors may limit the ability to compare the results of these instruments with other patient populations, comparisons within the population of patients with PKD are informative. Among adults, worse HRQoL and fatigue were consistently reported in patients receiving regular transfusions, those with pulmonary hypertension, and those with female gender. In FACT-An subscales, receipt of regular transfusions was associated with significantly lower physical, emotional, and social well-being, as well as increased symptoms of anemia. Worse HRQoL and fatigue was also generally associated with iron overload in both children and adults. Although iron chelation is associated with potential side-effects, lab monitoring, and costs, this finding may also be a marker of transfusion status. While symptoms within a patient are typically experienced at a certain hemoglobin level, this level may be different from patient to patient. Therefore, transfusions are not typically initiated based on a specific Hb level across the population, but rather improve that individual’s fatigue and HRQoL. However, patients who start regular transfusions may be more symptomatic and transfusions may not significantly improve HRQoL given the burden of time, energy, and cost placed on
the patient to undergo the intervention, and emphasizes the need for effective new therapies in this disease. The same overall principle—that more symptomatic patients are more likely to undergo splenectomy—may explain the finding that splenectomy was associated with higher fatigue in adults and worse HRQoL in children despite the fact that this is a one-time procedure performed to improve hemoglobin and reduce transfusion needs.

Pulmonary hypertension is a serious and relatively rare complication of PKD affecting 3% of individuals. Patients with this complication reported substantially reduced HRQoL and significantly increased fatigue. Physical and functional well-being were particularly impacted. Therefore, consideration of the possibility of pulmonary hypertension is critical in patients with concerning symptoms and demands aggressive management when identified.

Children, but not adults, reported significantly lower HRQoL with severe anemia (defined as a hemoglobin <8 g/dL). In the general PKD population, the relationship between hemoglobin and symptoms is not consistent between patients; however, in individual patients, there is often a relationship between hemoglobin level and symptoms including fatigue. Future studies with paired measurement of HRQoL, in patients before and after receiving therapy to raise their hemoglobin, will help to explore the relationship between hemoglobin and symptoms and capture the impact of improving the hemoglobin on HRQoL.

Additional differences in the clinical characteristics associated with reduced HRQoL and fatigue between children and adults included age, gender, and genotype. Older age was associated with reduced HRQoL in adults but not children. Reduced HRQoL with
advancing age is not unexpected generally, and especially in the population with PKD, as symptoms of the disease may become more pronounced with age-related declines in cardiopulmonary function, increasing co-morbidities, and emergence of time-dependent complications, such as bone disease and iron overload. In addition, female gender was associated with both reduced HRQoL and higher fatigue in adults but not children. Reduced HRQoL in female adult members of a given population as compared with male counterparts has been reported in a number of other chronic conditions and is not well-understood. This may be related to gender-based differences in reporting, higher rates of anxiety and depression in women which may result in lower HRQoL, or an inherent fault in the design of the instruments themselves, among other possibilities.

The presence of two severe mutations (non-missense/non-missense) is associated with worse anemia, more transfusion requirements, and more complications overall, and so it is expected that this would result in reduced HRQoL and increased fatigue. It is not clear why this correlation between genotype and HRQoL and fatigue was seen in children, but not adults, as the increased severity imparted by genotype persists throughout the lifespan.

While our study has a number of notable strengths, it also has several limitations. Although the sample size was large for this rare disease, the population was heterogeneous. The follow-up period was just two years and assessed patients at three time points. The instruments are generic and cancer-specific measures of HRQoL and fatigue likely insensitive to many of the unique aspects of PKD and congenital hemolytic anemias more generally. In this study, participants noted a significant impact of jaundice of HRQoL in a disease-specific assessment. This is notably absent from the
validated anemia questionnaires but represents an important symptom that future therapies should aim to improve. In patients with a congenital anemia, it may be difficult to assess fatigue or HRQoL except in the setting of a therapeutic intervention, such as pre/post splenectomy, pre/post transfusion, or pre/post a novel therapeutic. HRQoL instruments specific to the symptoms and impact of PKD have recently been developed and are presently being validated in phase III clinical trials of a novel therapy. If these new instruments perform well, they may be optimal for future studies evaluating HRQoL in PKD.

In conclusion, this first report of HRQoL and fatigue measurement in a large cohort of patients with PKD using validated, standardized instruments found similar HRQoL for patients with PKD as the general population but higher levels of fatigue. Female gender, receipt of regular transfusions, iron overload, and pulmonary hypertension were consistently associated with worse health-related outcomes in adults, while more severe anemia, iron overload, and two non-missense PKLR mutations were associated with worse patient-reported outcomes in children. Based on the findings of this study, future study of HRQoL in patients with PKD should utilize disease-specific instruments and evaluate the impact of therapeutic interventions on these outcomes.

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Table 1. Demographic characteristics of the PK deficiency cohort.

| Characteristics                  | All (N=254) |                |                |
|----------------------------------|-------------|----------------|----------------|
|                                  | n*          | % or median (range) |               |
| Gender                           | Male        | 124            | 48.9%          |
| Age at enrollment (y, range):    | Overall     | 254            | 19.0 (0.1-69.9)|
|                                  | < 18 years old | 123            | 6.4 (0.1-17.7)|
|                                  | ≥ 18 years old | 131            | 6.2 (18.0-69.9)|
| Race                             | Caucasian   | 235            | 92.5%          |
| Ethnicity                        | Hispanic    | 18             | 7.1%           |
| Amish                            |             | 55             | 21.7%          |
| Splenectomized                   |             | 150            | 59.1%          |
| Gallstones                       |             | 112/248        | 45.2%          |

| Characteristics                  | Children <18 years | Adults ≥18 years |
|----------------------------------|-------------------|-----------------|
|                                  | n*        | % or median (range) | n        | % or median (range) |
| Median number of lifetime transfusions | 86          | 18 (1-312)         | 63          | 39 (1-516)           |
| Genotype***                      | Mis sense/Mis sense | 55/97    | 57%           | 55/94          | 58%           |
|                                  | Mis sense/Non-Mis sense | 26/97      | 27%           | 25/94          | 27%           |
|                                  | Non-Mis sense/Non-Mis sense | 16/97       | 16%           | 14/94          | 15%           |
| Hemoglobin (g/dL)**              | Non-splenectomized NRT | 40          | 9.1 (6.0-12.5) | 30          | 11.3 (7.6-14.2) |
|                                  | Splenectomized NRT | 24          | 8.5 (4.3-12.8) | 52          | 8.5 (6.1-12.3) |
| Absolute Reticulocyte Count, (x10⁶ cells/µL) | 40          | 0.30 (0.07-5.36) | 42          | 0.21 (0.09-6.52) |
| Reticulocyte Percent (%)         | 87          | 11.2 (0.4-82.9)  | 54          | 18.9 (2.5-76)    |
| Bilirubin (mg/dl)                | 80          | 3.6 (0.1-33.1)   | 78          | 4.1 (0.9-17.6)   |
| Lactate Dehydrogenase (U/L)      | 46          | 775 (183-3811)   | 66          | 220 (127-1007)   |
| Ferritin (ng/ml)                 | 63          | 917 (22-13409)   | 72          | 594 (32-8220)    |

*Sample sizes are those with known data for the given characteristic from the Pyruvate Kinase Deficiency Natural History Study.

**NRT: not regularly transfused with <6 transfusions per year

***Those from the Amish community (homozygous R479H mutation) were excluded.
Table 2. Health Related Quality of Life survey results completed by age group at enrolment in patients with pyruvate kinase deficiency. SD, standard deviation

| Adult Surveys, ages ≥18 years (n=131) |  |
|--------------------------------------|--|
| EuroQoL-5D Visual Analog Scale        |  |
| Range 0 (worst) to 100 (best)        |  |
| n/N (%)                              | 120/131 (92%) |
| Median, range                        | 80, 20-100 |
| EuroQoL-5D Health Index Score*       |  |
| Range 0 (worst) to 1 (best)          |  |
| n/N (%)                              | 86/131 (66%) |
| Median, range                        | 0.88, 0.43-1 |
| FACT-An                              |  |
| Range 0 (most fatigue) to 188        |  |
| n/N (%)                              | 126/131 (96%) |
| Median, range                        | 156, 33-181 |
| PROMIS Fatigue 7a                    |  |
| (Mean 50, SD 10, higher is worse)    |  |
| n/N (%)                              | 60/131 (46%) |
| Median T score, range                | 52.3, 29.4-80.3 |

| Pediatric Surveys, age <18 years (n=123) |  |
|------------------------------------------|--|
| PedsQL 4.0                              |  |
| Range 0 (worst) to 100 (best)           |  |
| Parent Proxy                            |  |
| n/N (%)                                 | 86/123 (70.0%) |
| Median, range                           | 82.6, 33.7-100 |
| Child Self-Report                       |  |
| n/N (%)                                 | 61/123 (49.6%) |
| Median, range                           | 82.6, 33.7-100 |
| Peds-FACIT-F                            |  |
| Range 0 (most fatigue) to 52            |  |
| n/N (%)                                 | 50/123 (40.7%) |
| Median, range                           | 70.4, 31-80 |
| PROMIS Fatigue Child 10a                |  |
| (Mean 50, SD 10, higher is worse)       |  |
| Child Self-Report 10a                   |  |
| n/N (%)                                 | 36/123 (29.3%) |
| Median, range                           | 41.6, 30.3-65.7 |
| Parent Proxy Report 10a | 48/123 (39.0%) |
|------------------------|----------------|
| n/N (%)                | 46.3, 34.1-69.2|

Median, range

1 EuroQol-5D Health Index Score could only be analyzed for those participants from the United States, Germany, and the Netherlands
Table 3. Additional symptom assessment in children and adults with PK deficiency.

| Ages ≥18 years                                           | Total | Not at all | A Little bit | Somewhat | Quite a bit | Very much |
|----------------------------------------------------------|-------|------------|--------------|----------|-------------|-----------|
| I got upset about my jaundice (yellow eyes/skin)         | 68    | 28 (41.2%) | 18 (26.5%)   | 11 (16.2%) | 5 (7.4%)    | 6 (8.8%)  |
| Ages <18 years                                           | Total | None of the time | A Little of the time | Some of the time | Most of the time | All of the time |
| I got upset about my jaundice (yellow eyes/skin)         | 40    | 26 (65%)   | 7 (17.5%)    | 4 (10%)   | 1 (2.5%)    | 2 (5%)    |
| I had trouble walking                                    | 40    | 34 (85%)   | 2 (5%)       | 2 (5%)    | 2 (5%)      | 0         |
| I feel lightheaded (dizzy)                               | 41    | 26 (65%)   | 8 (20%)      | 7 (17.5%) | 0           | 0         |
| I have been short of breath                               | 40    | 24 (60%)   | 8 (20%)      | 7 (17.5%) | 1           | 0         |
| I have pain in my chest                                  | 40    | 32 (80%)   | 5 (12.5%)    | 3 (7.5%)  | 0           | 0         |
| I am less motivated to do my usual activities            | 40    | 26 (65%)   | 7 (17.5%)    | 7 (17.5%) | 0           | 0         |
**Table 4.** Patient characteristics significantly associated with reduced HRQoL or higher fatigue by HRQoL or fatigue instrument in adults (blue) and children (red). X, significant in entire cohort; X, significant only when Amish population excluded from analysis.

| Characteristic                        | EQ-5D | FACT-An | PROMIS-SF7a | PedsQL 4.0 | Peds-FACIT-F | PROMIS Child SF-10a |
|---------------------------------------|-------|---------|-------------|------------|--------------|---------------------|
| Older Age                             | X     |         |             |            |              |                     |
| Female Gender                         | X     | X       |             | X          |              |                     |
| Non-Amish                             | X     |         | X           |            |              |                     |
| Splenectomized                        | X     |         |             |            | X            |                     |
| Regularly Transfused                  | X     | X       | X           |            |              |                     |
| Iron Overload: Any Chelation Use      | X     |         |             |            | X            |                     |
| Iron Overload: Ferritin >1000 ng/mL   | X     |         |             |            | X            |                     |
| Pulmonary Hypertension                | X     |         |             |            | X            |                     |
| Non-Missense/Non-Missense Genotype    |       |         | X           |            |              |                     |
| Hemoglobin <8 g/dL                    |       |         |             | X          |              |                     |
| ≥18 total transfusions                |       |         |             |            | X            |                     |
FIGURES.

**Figure 1.** Report of HRQoL as assessed by the FACT-An in adults with PKD, by specific relevant subgroupings. Higher FACT-An scores indicate higher HRQoL. The R479H/R479H genotype is primarily found in the Amish community.

**Figure 2.** Report of fatigue as assessed by the Peds-FACIT-F in children with PKD, by specific relevant subgroupings. Higher Peds-FACIT-F scores indicate less fatigue.
