

# Prevalence of pyruvate kinase deficiency: A systematic literature review

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## Abstract

**Objectives:** Pyruvate kinase deficiency (PK deficiency) is a rare disorder caused by compound heterozygosity or homozygosity for > 300 mutations in the *PKLR* gene. To understand PK deficiency prevalence, we conducted a systematic literature review.

**Methods:** We queried Embase and Medline for peer-reviewed references reporting PK deficiency prevalence/incidence, *PKLR* mutant allele frequency (MAF) among the general population, or crude results from which these metrics could be derived.

**Results:** Of 1390 references screened, 1296 were excluded after title/abstract review; 60 were excluded after full-text review. Four of the remaining 34 studies were considered high-quality for estimating PK deficiency prevalence. Two high-quality studies identified cases from source populations of known sizes, producing estimates of diagnosed PK deficiency prevalence of 3.2 and 8.5 per million. Another high-quality study derived an estimate of diagnosed PK deficiency prevalence of 6.5 per million by screening jaundiced newborns. The final high-quality study estimated total diagnosed and undiagnosed PK deficiency prevalence to be 51 per million through extrapolation from observed MAFs.

**Conclusions:** We conclude that prevalence of clinically diagnosed PK deficiency is likely between 3.2 and 8.5 per million in Western populations, while the prevalence of diagnosed and undiagnosed PK deficiency could possibly be as high as 51 per million.

## KEYWORDS

incidence, PK deficiency, PKD, prevalence, pyruvate kinase deficiency, systematic literature review

## 1 | INTRODUCTION

Pyruvate kinase (PK) deficiency is a rare congenital disorder characterized by diminished activity of the PK enzyme in red blood cells (RBCs).<sup>1</sup> Low PK enzyme activity can lead to lifelong chronic hemolysis and associated symptoms and complications such as anemia,

jaundice, gallstones, thrombosis, and iron overload.<sup>2</sup> PK deficiency is a recessive disorder caused by compound heterozygosity or homozygosity for one or more of >300 known mutations<sup>3</sup> to the *PKLR* gene. The large number of *PKLR* mutations, which may have different impacts on PK function, likely contributes to the notable clinical heterogeneity of this disorder.<sup>2,4</sup>

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The prevalence of PK deficiency is unknown, with commonly cited estimates differing by orders of magnitude. Factors contributing to the uncertain prevalence include the extreme rarity of the disorder, the phenotypic similarity to other RBC disorders (leading to misdiagnosis), its heterogeneous clinical presentation, ethnic and geographic variability, and the current lack of adequate data from PK deficiency registries.

We conducted a systematic literature review (SLR) to assess the prevalence of PK deficiency and to better understand factors contributing to the wide range of reported values. We focused on studies reporting PK deficiency incidence, prevalence, survival duration, and/or mortality and studies reporting mutant allele frequencies (MAFs) for *PKLR* mutations among general populations.

## 2 | METHODS

A protocol containing the full details of study eligibility criteria, data to be extracted, and quality assessment process was created and maintained. We queried Embase and Medline via Ovid, searched the single conference year of the Deutsche Gesellschaft für Hämatologie und Medizinische Onkologie (DGHO) that was not indexed by Embase/Medline, and evaluated other relevant references encountered over the course of the SLR. Study eligibility was assessed by a review of titles and abstracts, followed by full-text review for potentially relevant studies. Data were extracted from studies meeting eligibility criteria, and quality assessment was performed using a custom tool. Study screening, data extraction, and quality assessment were conducted in duplicate by two independent reviewers, with disagreements resolved through consensus or referral to a third reviewer.

### 2.1 | Eligibility criteria

Peer-reviewed publications and conference abstracts were included if they were published (or in press) in English before January 23, 2019. Studies describing PK deficiency epidemiology and studies describing MAFs for *PKLR* mutations among general populations were included. We defined PK deficiency epidemiology studies as those reporting point/period prevalence, incidence rate/proportion, survival duration, life expectancy, and/or mortality rate among source populations selected without respect to PK deficiency symptoms. *PKLR* MAF studies were those that reported MAFs (number of alleles with a mutation out of total alleles) for mutations to the *PKLR* gene known to cause PK deficiency or predicted to significantly impair PK enzyme function. We also included studies reporting crude results from which PK deficiency epidemiology or *PKLR* MAFs could be derived.

We excluded studies that were not written in English, based on humans, subjected to peer-review, or were not the primary report of the data. We also excluded studies of PK deficiency prevalence/incidence conducted within a source population of patients with

### Novelty Statement

#### 1. What is the NEW aspect of your work? (ONE sentence)

This is a comprehensive and contemporary systematic review of studies of PK deficiency epidemiology which includes potential reasons for the observed variation in prevalence estimates.

#### 2. What is the CENTRAL finding of your work? (ONE sentence)

The prevalence of clinically diagnosed PK deficiency is likely between 3.2 and 8.5 per million in Western populations, while the prevalence of diagnosed and undiagnosed PK deficiency could possibly be as high as 51 per million.

#### 3. What is (or could be) the SPECIFIC clinical relevance of your work? (ONE sentence)

This work could help hematologists fully appreciate the potential for underdiagnosis and misdiagnosis of PK deficiency when diagnosing causes of hemolytic anemia, jaundice, and more.

symptoms of PK deficiency such as anemia or jaundice, as these populations would likely be enriched with patients with PK deficiency and not reflective of general populations. Similarly, *PKLR* MAF studies were excluded if they only reported MAFs among PK deficiency patients. We excluded studies reporting MAFs for only *PKLR* mutations unlikely to cause reduction of PK function as stated by the authors or inferred from amino acid substitution prediction models. Duplicate references were removed, though complementary studies with overlapping study populations were retained. Population, Intervention, Comparison, Outcomes and Study Design (PICOS) parameters were used to describe the eligibility criteria (Table S1).

### 2.2 | Information sources

#### 2.2.1 | Search

PK deficiency and *PKLR* Emtree and Medical Subject Heading (MeSH) terms and keywords were combined with epidemiology and gene frequency Emtree/MeSH terms and keywords in Ovid queries (Tables S2 and S3). The queries included references from January 1, 1974, (Embase) or January 1, 1946, (Medline) to January 23, 2019 (exclusive).

Conferences believed a priori to contain publications of PK deficiency epidemiology were indexed by Ovid except for a single conference year (2018) of the annual DGHO conference. The 2018



DGHO abstract booklet was searched for the terms “pyruvate kinase,” “PKD,” “PK deficiency,” and “PKLR.” Abstracts which contained  $\geq 1$  of these terms proceeded to title/abstract screening.

Finally, references encountered over the course of the literature review (eg, in the background section of another article) which appeared to be relevant were submitted to title/abstract screening. Each time a potentially relevant reference was encountered outside of the Ovid queries, screening terms were reevaluated.

## 2.2.2 | Study selection

Titles and abstracts of all references were screened, and those meeting inclusion/exclusion criteria were selected for full-text review. Data extraction and quality assessment were performed on studies meeting all eligibility criteria upon full-text review.

## 2.2.3 | Data extraction

Extracted data from eligible studies included the following: reference information, type and values of statistics reported, demographic and clinical characteristics of the study population, location of the study population, and study objectives and methods.

The primary outcome measure was PK deficiency prevalence. Where necessary, prevalence units were standardized. MAF estimates were converted to prevalence estimates assuming Hardy-Weinberg equilibrium (HWE) to facilitate comparison.

## 2.2.4 | Quality assessment and evidence synthesis

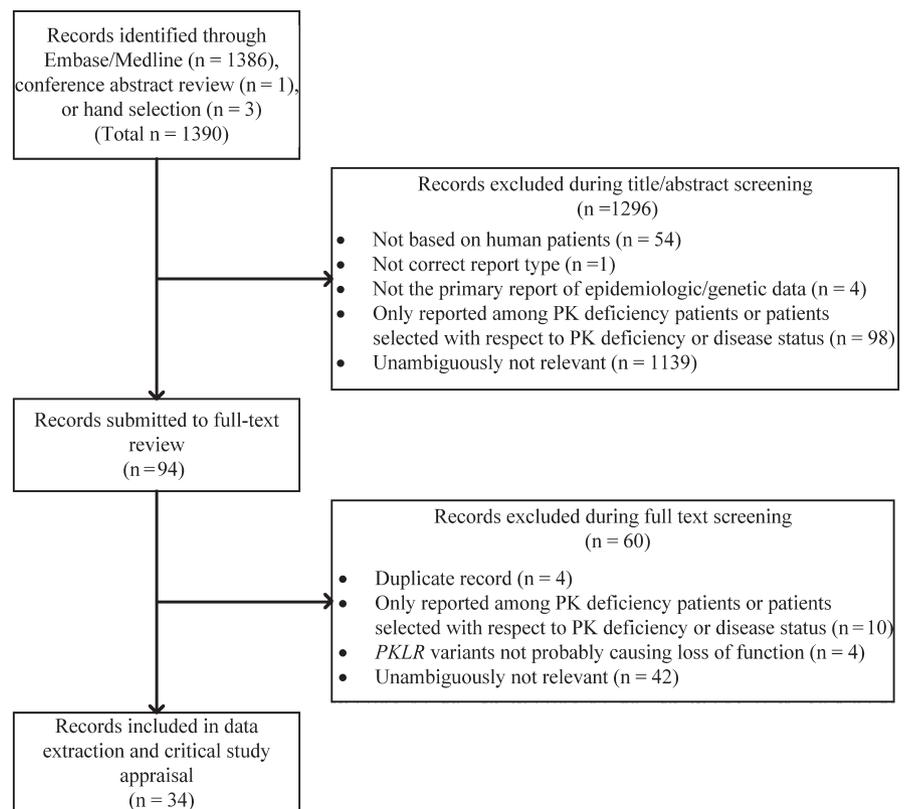
A tailor-made, qualitative, quality assessment tool was created based on the Joanna Briggs Institute critical appraisal tools<sup>5</sup> and the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) Statement.<sup>6</sup>

The generalizability, quality, and consistency of all outcome statistics were considered collectively to evaluate the weight and associated uncertainty of the evidence of PK deficiency prevalence.

## 3 | RESULTS

### 3.1 | Study selection and characteristics

The Ovid queries identified 1386 references (Tables S2 and S3). Additional potentially relevant references were identified from the 2018 DGHO conference abstract booklet ( $n = 1$ ) and during review of other references ( $n = 3$ ), for a total of 1390 references (Figure 1). Most studies were excluded during title/abstract screening ( $n = 1296$ ) and upon full-text review ( $n = 60$ ) due to irrelevance (Figure 1). To facilitate comparison, the remaining 34 studies meeting eligibility criteria were grouped as follows: population-based prevalence studies ( $n = 2$ ); molecular *PKLR* screening studies ( $n = 11$ ); and non-molecular PK deficiency screening studies ( $n = 21$ ). No studies reporting on mortality rates or life expectancy in PK deficiency were found.



**FIGURE 1** Flow diagram of studies meeting inclusion/exclusion criteria



Of the 34 studies meeting our inclusion criteria, four<sup>7-10</sup> were deemed to be high-quality for purposes of estimating overall disease prevalence.

### 3.2 | Results of population-based prevalence studies

Two of the four studies considered to be high-quality were population-based prevalence studies reporting counts of diagnosed PK deficiency cases out of source populations of known sizes (Table 1). Carey et al<sup>7</sup> estimated PK deficiency prevalence to be approximately 3.2 per million (Table 2) based on 10 cases diagnosed from 1974 to 1999 in a PK deficiency registry in the Northern Health Region of the United Kingdom among a general population of 3.1 million, while de Medicis et al<sup>8</sup> estimated PK deficiency prevalence to be 8.5 per million based on 56 confirmed cases with hemolytic anemia recorded in a hematologic database out of a population of 6.5 million individuals in Quebec. We consider these studies to have minimal risk of bias for the purpose of estimating PK deficiency prevalence given the large source populations and the implicit requirement for a clinician to suspect PK deficiency, though they likely underestimate PK deficiency prevalence by not accounting for undiagnosed cases.

### 3.3 | Results of molecular *PKLR* screening studies

Eleven studies screened individuals for mutations to the *PKLR* gene and reported MAFs (Table 1). However, many of these studies were uninformative because they identified *PKLR* mutations of unknown clinical significance in a general population without PK deficiency.

#### 3.3.1 | Molecular *PKLR* screening studies in a general population

Five studies evaluated *PKLR* mutations in general populations (Table 1). The most informative of these for our purposes, by Beutler and Gelbart,<sup>10</sup> utilized *PKLR* mutation data to estimate overall disease prevalence. This study was among the four in the review that we considered to be high-quality, in part because it addressed a potential source of bias present in other studies reporting on *PKLR* mutations. Namely, many molecular screening studies assumed that the penetrance of each mutation is 100%; that is, that each compound heterozygous or homozygous individual who carries a mutation will have symptoms of PK deficiency. This assumption may be incorrect, as the ability of a mutation to elicit clinical symptoms varies between individuals due to a variety of genetic and non-genetic factors.<sup>11</sup> Beutler and Gelbart<sup>10</sup> attempted to account for variable penetrance levels by utilizing an "index mutation," c.1529G > A, which they considered, based on clinical experience and its high frequency in a population with PK

deficiency, to have 100% penetrance. They assumed the ratio of this index mutation to other PK deficiency-causing mutations to be fixed and therefore to be equal between those with and without PK deficiency. Based on this assumption and MAFs from 20 PK deficiency cases and 3785 non-cases, they derived an estimate of PK deficiency prevalence of 51 per million, albeit with a substantial amount of associated uncertainty (standard error = 32.5 per million). Notably, this estimate represents the prevalence of diagnosed and undiagnosed cases and so is distinct from the previously described population-based studies which estimated the prevalence of diagnosed PK deficiency cases only. However, the accuracy of this estimate depends on the true distributions of *PKLR* mutations between those with and without PK deficiency, which are not fully known.

All of the other studies reporting on *PKLR* MAFs among general populations did not consider the full suite of known mutations to the *PKLR* gene<sup>10,12,13</sup> or included mutations that may not cause loss of PK function and, therefore, are less useful for our purposes.<sup>14</sup> All determined MAFs for the *PKLR* mutation c.1456C > T, one of the most common mutations in European patients with PK deficiency.<sup>15</sup> In the largest of these,<sup>16</sup> Chami and colleagues reported the MAF of c.1456C > T<sup>a</sup> to be 0.30% among up to 130 273 individuals. *PKLR* MAFs derived in the other four studies were generally consistent with this finding, reporting MAF ranges from 0.10%<sup>13</sup> to 0.33%.<sup>12</sup>

In the only study to fully sequence the *PKLR* gene, Figueroa et al<sup>14</sup> reported a MAF for all *PKLR* mutations to be 1.09%, a high MAF that likely relates to the inclusion of mutations not known to cause loss of function.

#### 3.3.2 | Molecular *PKLR* screening studies in areas with endemic malaria

Four studies screened for *PKLR* variants in areas with endemic malaria to evaluate the hypothesis that malaria induces a selection pressure that increases PK deficiency prevalence which, if true, would undermine the generalizability of such studies (Table 1). Of the four studies, two did not identify any *PKLR* mutations in their study populations,<sup>17,18</sup> while two others found high MAFs between 1.50%<sup>19</sup> and 4.6%.<sup>20</sup> These studies are limited by the uncertainty around the clinical impact of the reported *PKLR* mutations.

#### 3.3.3 | Molecular *PKLR* screening studies in other unique populations

One study<sup>21,22</sup> used whole-exome sequencing to investigate the heritability of attention-deficit hyperactivity disorder. It was found incidentally that a child among four family members whose exomes were sequenced was a compound heterozygote for *PKLR* mutations. Though this study met our inclusion criteria, it is uninformative of PK deficiency prevalence.

TABLE 1 Summary table of studies selected for data extraction

First author (year) <sup>Citation</sup>	Study purpose	Source population
<b>Population-based prevalence study</b>		
Carey (2000) <sup>7</sup>	To estimate the prevalence of PK deficiency in a general, white population	Former Northern Health Region of the United Kingdom
de Medicis (1992) <sup>8</sup>	To estimate the prevalence of PK deficiency associated with hemolytic anemia in Quebec	Quebec, Canada
<b>Molecular PKLR screening study in a general population</b>		
Beutler (2000) <sup>10</sup>	To estimate the prevalence of PK deficiency in a general, predominately white population	3785 anonymous individuals without PK deficiency in the USA, predominately white; 20 patients with PK deficiency, mostly white Americans
Chami (2016) <sup>16</sup>	To identify pleiotropic genetic mutations associated with RBC traits	Up to 130 273 individuals in 24 discovery cohorts and five ancestries: European, African American, Hispanic, East Asian, and South Asian ancestries
Figuroa (2018) <sup>14</sup>	To evaluate whether genetic mutations in kinases affect Tenofovir's efficacy in the treatment/prevention of HIV	505 individuals in HIV prevention trials in Thailand, the USA, and South Africa
Manco (2001) <sup>12</sup>	To determine the frequency of allele 1456C-T in a general population in Portugal	616 unrelated Portuguese individuals
Svidnicki (2018) <sup>13</sup>	To evaluate PKLR mutations among Brazilian patients with PK deficiency and healthy individuals	500 healthy Brazilian blood donors
<b>Molecular PKLR screening study in areas with endemic malaria</b>		
Alves (2010) <sup>18</sup>	To evaluate the associations between malaria and sickle cell disease, G6PD, and PK deficiency	257 unrelated samples (selected from 1056 total samples) from Santiago Island, Ecuador
Berghout (2012) <sup>19</sup>	To evaluate the frequency of PKLR mutations to address whether PKLR mutations are protective in malaria-pressured areas	387 unrelated individuals, primarily from African and Asian origin
Machado (2010) <sup>17</sup>	To evaluate possible associations between PKLR variants and malaria	396 individuals, 316 from Africa (including malaria-infected and uninfected children) and 80 from Portugal
Machado (2012) <sup>20</sup>	To determine PK deficiency prevalence in sub-Saharan Africa, to evaluate PKLR variant frequency, and to evaluate the PK deficiency-malaria association	611 individuals in sub-Saharan Africa for gene frequency analysis, 296 for enzyme assays
<b>Non-molecular PK deficiency screening study in a general population</b>		
Feng (1993) <sup>32</sup>	To establish a reference interval of PK activity and to determine PK deficiency prevalence in local Chinese population	497 healthy men and 100 neonates in China
Fox (1969) <sup>33</sup>	To evaluate two different screening methods for erythrocyte enzyme deficiencies	1000 blood samples from individuals in a general hospital in the USA
Fung (1969) <sup>31</sup>	To determine the incidence of PK deficiency and G6PD deficiency in the Chinese newborn in Hong Kong and the possible relation of PK deficiency with neonatal jaundice	700 consecutive neonates in Hong Kong
Garcia (1979) <sup>28</sup>	To estimate prevalence of enzyme deficiencies including PK deficiency in a Spanish population	1636 hematologically normal individuals in Spain
Mohrenweiser (1981) <sup>34</sup>	To evaluate the frequency of genetic variants resulting in total or near total loss of enzyme activity for nine human erythrocyte enzymes	675 neonates and 200 of their parents in the USA (697 total assayed for PK deficiency)

(Continues)



TABLE 1 (Continued)

First author (year) <sup>Citation</sup>	Study purpose	Source population
Mohrenweiser (1987) <sup>35</sup>	To estimate the frequency of enzyme deficiency variants including PK deficiency	2020 unrelated neonates in the USA
Tanaka (1971) <sup>30</sup>	To offer a thorough description of PK diagnosis and epidemiology	146 normal individuals in the USA
Tsang (1993) <sup>27</sup>	To evaluate a new PK enzyme activity assay	193 healthy adults in Hong Kong
Wu (1985) <sup>29</sup>	To estimate the prevalence of PK deficiency in a Chinese population of clinically normal neonates	1159 random clinically normal infants from China
<b>Non-molecular PK deficiency screening study in areas with endemic malaria</b>		
El-Hazmi (1986) <sup>36</sup>	To estimate PK deficiency and hexokinase deficiency in a population with a high frequency of G6PD deficiency	3120 individuals in outpatient clinics in Saudi Arabia
Munyanganizi (2006) <sup>37</sup>	To determine the prevalence of hemoglobin variants including PK deficiency in Rwanda	987 neonates in Rwanda
Upadhye (2018) <sup>38</sup>	To estimate the prevalence of hemoglobinopathies and enzyme deficiencies in India	2400 neonates in India
<b>Non-molecular PK deficiency screening study in areas with high consanguinity</b>		
Abu-Melha (1991) <sup>44</sup>	To explore the prevalence of PK deficiency in Saudis	513 clinically normal neonates in Saudi Arabia
Akin (1997) <sup>41</sup>	To explore the prevalence of PK deficiency in a Turkish population	1190 unrelated individuals from Turkey
Al-Naama (1994) <sup>42</sup>	To explore erythrocyte activities including PK deficiency in Iraq	1226 clinically normal neonates and 498 healthy students in Iraq (n = 1724 total)
Al-Naama (1995) <sup>43</sup>	To estimate red blood cell enzyme deficiencies including PK deficiency in a population with high G6PD deficiency prevalence	506 clinically normal neonates and 343 healthy adults (n = 849 total)
Christensen (2010) <sup>9</sup>	To report on neonates with PK deficiency, encourage clinicians to consider PK deficiency diagnosis, and discuss PK deficiency in a polygamous community	153 830 neonates screened for jaundice in the USA
Yavarian (2008) <sup>40</sup>	To estimate PK deficiency prevalence in a south Iranian population	4017 randomly selected individuals from Iran
<b>Non-molecular PK deficiency screening in other unique population</b>		
Mohrenweiser (1984) <sup>45</sup>	To evaluate the prevalence of rare electrophoretic mobility variants including PK deficiency among Native Americans	Approximately 10% of 629 Native Americans
Satoh (1983) <sup>46</sup>	To explore enzyme activity including PK in children of parents exposed to atomic bomb radiation in Japan	3069 children of individuals exposed to atomic bomb radiation in Japan
Satoh (1985) <sup>47</sup>	To explore the frequency of inherited variations in enzyme thermostability including PK within a population of children of atomic bomb survivors	974 children of individuals exposed to atomic bomb radiation in Japan
<b>Molecular PKLR screening in other unique population</b>		
Lyon (2011) <sup>22</sup>	To explore the hypothesis that rare family specific genetics may account for the remaining missing heritability of ADHD	A family of 4 of whom 3 have ADHD
Robinson (2010) <sup>21</sup>	To explore the hypothesis that rare family specific genetics may account for the remaining missing heritability of ADHD	A family of 4 of whom 3 have ADHD

Abbreviations: ADHD, attention-deficit hyperactivity disorder; G6PD, glucose-6-phosphate dehydrogenase; HIV, human immunodeficiency virus; PK deficiency, pyruvate kinase deficiency; PK, pyruvate kinase; RBC, red blood cell; USA, United States of America.



TABLE 2 Summary of results by study type

First author (year) <sup>Citation</sup>	PKLR genes sequenced	Reported results	Prevalence estimates (per million)	Derivation
<b>Population-based prevalence study</b>				
Carey (2000) <sup>7</sup>	N/A	Period prevalence: 3.2 per million	3.2	†
de Medicis (1992) <sup>8</sup>	N/A	Period prevalence: 1 per 117 206	8.5	‡
<b>Molecular PKLR screening study in a general population</b>				
Beutler (2000) <sup>10</sup>	c.1456C > T, c.1468C > T, c.1484C > T, c.1529G > A	Estimated prevalence: 51 per million	51	†
Chami (2016) <sup>16</sup>	c.1456C > T	MAF for 1 PK deficiency-causing allele: 0.30%	9.0	§
Figueroa (2018) <sup>14</sup>	Whole PKLR gene	MAF of 1.1%	120	§
Manco (2001) <sup>12</sup>	c.1456C > T	MAF of single allele: 0.325% (95% CI 0.001% to 0.649%) Estimated point prevalence: 10 per million	10	†
Svidnicki (2018) <sup>13</sup>	c.1456C > T, c.1529G > A	MAF for 2 common alleles: 0.10%	1	§
<b>Molecular PKLR screening study in areas with endemic malaria</b>				
Alves (2010) <sup>18</sup>	c.269T > A and c.1456C > T	MAF for two alleles: 0.0%	0	N/A
Berghout (2012) <sup>19</sup>	Whole PKLR gene	MAF: 1.5% (SD: 0.9%) Estimated point prevalence of PK deficiency: 1/4203 births assuming HWE	240	‡
Machado (2010) <sup>17</sup>	c.1456C > T and c.1614A > T	MAF for two alleles: 0.0%	0	N/A
Machado (2012) <sup>20</sup>	Whole PKLR gene	MAF for 1 allele in children: 2.9% MAF for 1 allele in adults: 4.6% Low PK activity prevalence (Non-molecular PK deficiency screening): 4.1%	840 (children) <sup>§</sup> ; 2100 (adults) <sup>§</sup> ; 41 000 (Non-molecular PK deficiency screening) <sup>‡</sup>	‡, §
<b>Non-molecular PK deficiency screening study in a general population</b>				
Feng (1993) <sup>32</sup>	N/A	Point prevalence: 0.0%	0	N/A
Fox (1969) <sup>33</sup>	N/A	Point prevalence: 0.0%	0	N/A
Fung (1969) <sup>31</sup>	N/A	Incidence/point prevalence in newborns (partial and total deficiency): 3.4% Incidence/ point prevalence in newborns (partial deficiency): 2.3% Incidence/point prevalence in newborns (total deficiency): 1.1%	34 000 <sup>‡</sup> (partial and total deficiency) 23 000 <sup>‡</sup> (partial deficiency) 11 000 <sup>‡</sup> (total deficiency)	‡
Garcia (1979) <sup>28</sup>	N/A	Point prevalence: 0.24%	2400 <sup>‡</sup>	‡
Mohrenweiser (1981) <sup>34</sup>	N/A	Incidence/point prevalence in newborns: 0.14%	1400 <sup>‡</sup>	‡
Mohrenweiser (1987) <sup>35</sup>	N/A	Incidence/ prevalence at birth using ≤ 65% normal activity threshold: 1.1% Incidence/prevalence at birth using < 50% normal activity threshold: 0.50%	11 000 <sup>‡</sup> 5000 <sup>‡</sup>	‡
Tanaka (1971) <sup>30</sup>	N/A	Point prevalence: 1.4%	14 000 <sup>‡</sup>	‡
Tsang (1993) <sup>27</sup>	N/A	Point prevalence using modified technique: 0.0% Point prevalence using Beutler technique: 3.7%	0 (modified technique) 37 000 (Beutler technique)	‡
Wu (1985) <sup>29</sup>	N/A	Incidence/prevalence at birth (Beutler spot test): 2.2% Incidence/prevalence at birth (direct enzyme activity): 2.1%	22 000 (Beutler spot test) 21 000 (enzyme activity screening)	‡

(Continues)



TABLE 2 (Continued)

First author (year) <sup>Citation</sup>	PKLR genes sequenced	Reported results	Prevalence estimates (per million)	Derivation
<b>Non-molecular PK deficiency screening study in areas with endemic malaria</b>				
El-Hazmi (1986) <sup>36</sup>	N/A	Point prevalence of partial PK deficiency: 3.4% Prevalence of complete PK deficiency: 0.0%	34 000 (partial) 0 (complete)	†
Munyanganizi (2006) <sup>37</sup>	N/A	Point prevalence: 0.0%	0	N/A
Upadhye (2018) <sup>38</sup>	N/A	Point prevalence: 0.083%	830	††
<b>Non-molecular PK deficiency screening study in areas with high consanguinity</b>				
Abu-Melha (1991) <sup>44</sup>	N/A	Incidence/prevalence among neonates by fluorescence screening: 2.90% Incidence/prevalence among neonates by fluorescence screening and enzyme activity screening: 3.10% (95% CI: 1.5)	29 000 (fluorescence screening) 31 000 (fluorescence screening and enzyme activity)	‡
Akin (1997) <sup>41</sup>	N/A	Point prevalence of "heterozygous deficiency": 1.10% Point prevalence of "homozygous deficiency": 0.00%	11 000 0	‡
Al-Naama (1994) <sup>42</sup>	N/A	Point prevalence of PK deficiency: 0.0%	0	N/A
Al-Naama (1995) <sup>43</sup>	N/A	Point prevalence of partial PK deficiency among neonates: 1.4% Point prevalence of partial PK deficiency among adults: 0.0%	14 000 (partial PK deficiency among neonates) 0 (partial PK deficiency among adults)	‡
Christensen (2010) <sup>9</sup>	N/A	Point prevalence of partial PK deficiency among all age groups: 0.82% Point prevalence of PK deficiency among all age groups: 0.0% Incidence proportion/prevalence at birth of hospital including polygamist community: 1/30 000	8200 (partial PK deficiency among all age groups) 0 (complete PK deficiency) 33 (total population)	‡
Yavarian (2008) <sup>40</sup>	N/A	Point prevalence based on 60% PK activity: 1.9% Point prevalence of homozygous individuals: 0.27%	19 000 2700	‡
<b>Non-molecular PK deficiency screening in other unique population</b>				
Mohrenweiser (1984) <sup>45</sup>	N/A	Point prevalence: 0.0%	0	N/A
Satoh (1983) <sup>46</sup>	N/A	Mutant allele frequency for all alleles: 1.4%	14 000	‡
Satoh (1985) <sup>47</sup>	N/A	Mutant allele frequency for all alleles: 0.0%	0	N/A
<b>Molecular PKLR screening in other unique population</b>				
Lyon (2011) <sup>22</sup>	Whole PKLR gene	Point prevalence: 1 PK deficiency patient out of 4 individuals studied = 25%	250 000	‡‡
Robinson (2010) <sup>21</sup>	Whole PKLR gene	Point prevalence: 1 PK deficiency patient out of 4 individuals studied = 25%	250 000	‡‡

Abbreviations: HWE, Hardy-Weinberg equilibrium; MAF, mutant allele frequency; PK deficiency, pyruvate kinase deficiency; PK, pyruvate kinase.

†Estimate provided by study authors.

‡Units converted from authors' estimate.

‡Estimate derived assuming HWE.

††Average partial PK deficiency prevalence calculated from the individual estimates for each location.

‡‡Derived from two patients with PK deficiency among 2400 neonates.

‡‡Derived units from 1 PK deficiency case among 4 individuals sequenced.



### 3.4 | Results of non-molecular PK deficiency screening studies

Twenty-one studies used enzymatic assays to identify individuals with low PK activity. These studies have a high risk of bias due to the poor accuracy of screening assays, which can be subjective,<sup>23</sup> incomparable prior to standardization,<sup>24,25</sup> not always reflective of *in vivo* PK function,<sup>26</sup> and sensitive to experimental procedures.<sup>27</sup> In particular, PK activity assays may overestimate PK deficiency prevalence because of incomplete lysis of RBC or underestimate PK deficiency prevalence due to incomplete white blood cell or platelet removal or blood donor contamination in a transfused case. PK activity assays may also fail to distinguish between heterozygotes and compound heterozygotes or homozygotes, leading to inflated estimates of PK deficiency prevalence.

#### 3.4.1 | Non-molecular PK deficiency screening studies in a general population

Three studies screened for patients with PK deficiency among populations considered by the authors to be “normal” or “healthy,” observing PK deficiency prevalence values between 0.2% and 2.2%.<sup>28–30</sup> Given the extremely high prevalence values reported in these “healthy” populations, which do not comport with clinician experience of PK deficiency as a rare condition, it seems particularly likely that they are subjected to diagnostic limitations. Indeed, Tsang et al<sup>27</sup> argue that incomplete RBC lysis may produce artificially low PK activities and demonstrated in samples from 493 healthy adults that simply freezing RBC to promote lysis can reduce PK deficiency prevalence estimates from 3.7% to 0.0%.

Six enzyme screening studies that did not restrict to “normal” or “healthy” individuals may have contained similar diagnostic issues. In a study of 700 consecutive newborns in Hong Kong, the PK deficiency prevalence was observed to be 1.1% (total PK deficiency) and 3.4% (total and partial PK deficiency).<sup>31</sup> Notably, all eight newborns with total PK deficiency were jaundiced, lending credibility to the observed prevalence of PK deficiency. However, the extremely high prevalence merits increased scrutiny on the diagnostic methods, which were qualitative in nature and potentially unblinded to each individual's jaundice status.

One study by Feng and colleagues was coauthored by Tsang subsequent to her finding that incomplete RBC lysis may cause false-positive results for PK deficiency in the enzyme assay.<sup>32</sup> This study, conducted among 497 healthy men and 100 neonates, found no cases of PK deficiency using a method that included freezing to promote lysis.<sup>25</sup> In another study, by Fox and colleagues,<sup>33</sup> no patients with PK deficiency were detected among 1000 individuals in the United States in a diverse population.

Mohrenweiser reported on a screening study of newborn infants at the University of Michigan in two manuscripts. In the first,<sup>34</sup> 675 newborns and 200 adults were assayed for enzyme activity (697 total for PK activity) using a unique method which required presumptive cases to be confirmed in separate assays. With this method, one PK

deficiency case out of 697 individuals was detected for a prevalence of 0.14%. This relatively high estimate of PK deficiency prevalence may reflect the true PK deficiency prevalence in the study population and/or random sampling with a small population: The lower bound of the 95% confidence interval (CI) is 37.5 cases per million, which is more consistent with other studies. In the other study on an overlapping population,<sup>35</sup> 2013 unselected newborns were screened for PK deficiency. Using a threshold of <50% of normal activity to define PK deficiency, only one PK deficiency case was identified (0.50%). Again, small sample size may have inflated the impact of a single PK deficiency case (lower 95% CI: 12.6 cases per million).

#### 3.4.2 | Non-molecular PK deficiency screening studies in areas with endemic malaria

We identified three studies in areas with endemic malaria that screened for PK deficiency with enzymatic techniques (Table 1). Given our concerns for enzyme assay accuracy and the poor generalizability of populations in malaria-endemic regions, the risk of bias for these studies was considered to be high. These studies produced estimates of complete PK deficiency of 0.0%<sup>36,37</sup> to 0.083%.<sup>38</sup>

#### 3.4.3 | Non-molecular PK deficiency screening studies in areas with high consanguinity

Six studies investigated PK deficiency prevalence in areas with high consanguinity (Table 1). As with other studies based on enzyme assays and in consanguineous populations, these must be interpreted with caution for concerns related to assay accuracy and study population generalizability.

Despite these concerns, one study, by Christensen and colleagues,<sup>9</sup> was deemed to be of high-quality and useful for the estimation of PK deficiency prevalence. The authors screened 153 830 live births in the USA for high bilirubin; those with neonatal jaundice were subsequently tested for PK deficiency. Five neonates were found to have PK deficiency (prevalence of approximately 1 case per 30 000 births); however, the authors speculated that a polygamous Amish community with a known founder effect<sup>39</sup> may be responsible for four of the five PK deficiency cases (prevalence of one case per 250 births in the polygamous community). The implied prevalence in the non-polygamous community is one birth per 152 830 births or 6.5 per million. We considered this study design to have a low risk of bias because neonates required clinical suspicion of PK deficiency and confirmatory symptoms (ie, jaundice); however, the estimation of the number of births from the purportedly polygamous community may be subjected to error.

In another study by Yavarian et al,<sup>40</sup> 4017 Iranian individuals were screened for low PK activity (<60% normal). The 74 individuals (1.9%) with low PK activity were then evaluated for PKLR mutations. Eleven individuals possessed two PKLR mutations (0.27% of



the 4017 screened individuals). Because the authors used both molecular and enzymatic methods to identify PK deficiency cases, we considered the diagnoses of PK deficiency to have a low risk of bias. However, the study region has a high rate of consanguinity, limiting the generalizability of this study.

In a third study by Akin et al,<sup>41</sup> the authors found the heterozygous deficiency (<60%) prevalence to be 1.1% and the homozygous deficiency (<30% normal activity) prevalence to be 0.0%.

The three remaining non-molecular PK deficiency screening studies in areas with high consanguinity reported populations described as “clinically normal” or “healthy,” further limiting their generalizability. The prevalence estimates in these studies ranged from 0.0%<sup>42,43</sup> to 3.1%.<sup>44</sup>

### 3.4.4 | Non-molecular PK deficiency screening studies in other unique populations

Three studies used non-molecular techniques to screen for PK deficiency in unique and potentially non-generalizable populations. Mohrenweiser et al<sup>45</sup> screened approximately 10% of 629 Native American individuals who the study's authors suspected may have abnormally high frequencies of PK deficiency; however, none had PK deficiency. Two studies with overlapping study populations by Satoh et al<sup>46,47</sup> screened children of survivors of the atomic bombs in Japan and found prevalence values of 1.4% (threshold < 66% of normal) among 3069 children<sup>46</sup> and 0.0% (via a thermostability assay) among 974 children.<sup>47</sup>

## 4 | DISCUSSION

Identified studies varied widely in their reported or implied prevalence of PK deficiency. Much of this variation was attributable to known factors, such as the generalizability of source populations. Studies estimating prevalence in malaria-endemic areas, among populations with high consanguinity or among “healthy,” asymptomatic patients, would not be expected to be applicable to a general population. PK activity assay methods likely played a large role in the heterogeneity of PK prevalence values as well, as these methods can be subjective (ie, visual examination), sensitive to chosen PK activity thresholds, and sensitive to experimental conditions. Similarly, studies of *PKLR* MAFs may not be indicative of disease prevalence due to the heterogeneous effects of different *PKLR* mutations. Finally, the definition of PK deficiency was a major source of heterogeneity between identified studies. Some studies reported only on clinically diagnosed cases; these studies would be expected to underestimate the true prevalence of PK deficiency by omitting undiagnosed cases. Others attempted to estimate the prevalence of diagnosed and undiagnosed PK deficiency.

We considered four studies to provide high-quality estimates of PK deficiency prevalence. The population-based studies reported prevalence values of 3.2 per million<sup>7</sup> and 8.5 per million.<sup>8</sup> Additionally,

although the Christensen study<sup>9</sup> investigated an area with high consanguinity due to a polygamous population, the implied prevalence among non-polygamous individuals was considered to be a high-quality estimate (6.5 per million). As discussed previously, these studies likely underestimated PK deficiency prevalence because they considered only diagnosed and symptomatic clinical cases.

The Beutler and Gelbart study estimated diagnosed and undiagnosed PK deficiency prevalence to be 51 per million<sup>10</sup> and was deemed to have a low risk of bias. Most studies sequencing *PKLR* to estimate PK deficiency prevalence or MAFs contained two competing sources of bias: the measurement of only a subset of *PKLR* mutations (leading to prevalence underestimates) and the potential for some measured *PKLR* mutations to not cause PK deficiency or to not occur in PK deficiency patients at the same frequency as in the general population (leading to prevalence overestimates). The Beutler and Gelbart study is the only one that attempted to address both limitations. Notably, due to the small counts of patients with PK deficiency, the standard error of the reported prevalence estimate was high (32.5 per million), suggesting a large degree of uncertainty around the point estimate of 51 per million. An additional source of uncertainty in the Beutler and Gelbart estimate results from the unclear validity of the assumption that PK deficiency cases and non-cases have the same distribution of *PKLR* mutations that impair PK enzyme function.

## 5 | CONCLUSIONS

The prevalence of PK deficiency in general Western populations is likely greater than 3.2-8.5 per million and may be as high as 51 per million. Future studies are needed to understand the clinical significance of various mutant alleles, which may inform more accurate, clinically relevant PK deficiency prevalence estimates, identify the degree of and reasons for underdiagnosis, and elucidate PK deficiency heterogeneity between populations. Some of this additional research is currently being conducted through existing patient registries.

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### CONFLICT OF INTEREST

CC and DC are employees of IQVIA. IQVIA received research funding from Agios Pharmaceuticals to conduct this study. KG, MS, LP, and ANB are employees of Agios pharmaceuticals. MHS has no potential conflicts of interest to declare.

### AUTHOR CONTRIBUTIONS

MHS drafted the manuscript. MS, CC, DC, KG, LP, and ANB provided critical revisions to the manuscript. MHS, MS, CC, DC, and ANB designed and executed the study. MS and ANB conceived of the study. All authors approved of the final manuscript version.



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## ENDNOTE

<sup>a</sup> In the study by Chami et al, this mutation was incorrectly referred to as c.1456T > G.

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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