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RESEARCH ARTICLE

10-Year Risk of Gallstones in Congenital Red Blood Cell Disorder Patients: A Nationwide Cohort Study

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ABSTRACT

Chronic hemolysis potentially elevates the risk of gallstones in several types of congenital red blood cell (RBC) disorders. However, the magnitude of the risk is unknown. We investigate the risk of gallstone disease in congenital RBC disorder patients, compared with general population comparators. Patients were identified from the Danish National Patient Registry covering all Danish hospitals and the National Reference Laboratory for RBC disorders during 1980–2016. Patients were matched by sex, age, and region of origin with up to 50 general population comparators. Gallstone events were identified using hospital-registered diagnoses and surgery codes. Our study included 9354 congenital RBC disorder patients, grouped according to type of congenital RBC disorder, and 416 994 general population comparators. The cumulative 10-year incidence of gallstone disease was 4.2% in patients with congenital RBC disorders and 1.7% among comparators. Adjusted csHR's [95% confidence interval] were 8.1 [6.8, 9.7] for hereditary spherocytosis; 3.3 [1.6, 6.8] for glucose-6-phosphate dehydrogenase deficiency; 21.6 [10.6, 44.1] for pyruvate kinase deficiency; 3.7 [1.9, 7.0] for sickle cell disease; 0.8 [0.4, 1.6] for sickle cell trait; 1.5 [1.1, 2.2] for α-thalassemia trait; 1.8 [1.4, 2.3] for β-thalassemia minor; and 2.1 [1.8, 2.6] for other congenital hemolysis. We found a markedly higher risk of hospital-registered gallstone diseases in nearly all groups of patients with congenital RBC disorders compared with the general population.

1 | Introduction

Congenital hemolytic anemias are red blood cell (RBC) disorders in which deficiency of hemoglobin, RBC enzymes, or membrane and cytoskeletal proteins lead to premature RBC destruction, that is, chronic hemolysis [1]. These disorders are increasingly prevalent even in nonendemic countries as a result of global population movements. They are an increasing public health concern due to morbidity and, for some types, reduced life expectancy [2–5]. Chronic hemolysis often causes hyperbilirubinemia, thus potentially promoting the formation of black pigment gallstones. Prior studies have shown that various types of congenital RBC disorders are frequently associated with gallstones, and the rate of occurrence often increases with increasing hemolytic intensity [6–9]. However, even in the general adult population the prevalence of gallstones is 10%–15%, with black pigment gallstones accounting for only 5%–10% of cases [10]. Although one prior study examined gallstone prevalence in women with thalassemia minor and in a comparison group [7], it is not well established whether, and to what extent, congenital RBC disorders are associated with elevated risk of gallstone disease [11, 12]. For patients with congenital RBC disorders, existing frequency data primarily stem from small cross-sectional studies and

retrospective cohorts that used ultrasound or journal data to estimate gallstone prevalence/incidence regardless of symptoms [6–9, 13–16]. Because as many as 80% of gallstones remain asymptomatic, overall prevalence may have low clinical relevance [17]. In this study, we investigated the risk of hospital-diagnosed gallstone disease in patients with congenital RBC disorders, compared with a matched cohort drawn from the general population. We examined for the first time the magnitude of risk of gallstone disease among patients with congenital RBC disorders.

2 | Methods

2.1 | Study Design

Denmarks' almost 6 million inhabitants have a free tax-supported healthcare system [18, 19]. We established a nationwide cohort to study the risk of hospital-diagnosed gallstone events (gallstone-related disease or surgery) among patients with congenital RBC disorders. In addition, we performed a systematic review of available published data. The methods and results of this review are described in the Supporting Information S1 and Supplementary Figure 1.

2.2 | Setting, Participants, and Outcomes

Our study cohort encompassed all patients with a first ever diagnosis of congenital RBC disorder identified from the Danish Hemolysis Cohort. This nationwide cohort includes all patients in Denmark with an RBC disorder diagnosed between 1980 and 2016 [2, 20–22]. The cohort was constructed using patients identified from the Danish National Patient Registry (DNPR) covering all Danish hospitals, supplemented with patients diagnosed at the National Reference Laboratory for RBC disorders, Department of Hematology, Rigshospitalet [2, 21]. The DNPR records all patients treated in Danish public hospitals since 1977, with diagnoses coded according to the *International Classification of Diseases, Eighth Revision* (ICD-8) through 1993 and the *Tenth Revision* (ICD-10) thereafter. The DNPR includes information on discharged diagnosis (primary diagnosis and up to 20 contributing diagnoses) and registration on surgical procedures performed during the hospitalization or outpatient clinic visit [20].

During the 1977–1993 period, the DNPR captured diagnoses only from inpatient hospitalizations. Since 1994, diagnoses from outpatient specialist clinic and emergency department visits also have been available [20]. Patients joined the Danish Hemolysis Cohort on the diagnosis date of a congenital RBC disorder, including hereditary spherocytosis (HS), enzyme disorder (glucose-6-phosphate dehydrogenase deficiency [G6PD] and pyruvate kinase deficiency [PKD]), hemoglobin disorders (sickle cell disease [SCD], sickle cell trait [SCT], α-thalassemia trait, or β-thalassemia minor), and other RBC disorders (primarily a large group of unspecified congenital RBC disorders and a few rare congenital RBC disorders) (Table S1) [2].

Each patient was matched with up to 50 comparators from the general population, based on year of birth, sex, and global region of origin. Comparators were assigned the same follow-up start date as patients.

The primary outcome was hospital-registered gallstone events defined as a composite outcome comprising any discharged diagnosis (primary or contributing) associated with gallstone and/or surgery registration of gallstone-related procedures, see Table S2 for specific codes used for defining outcome. Due to the higher granularity in the ICD-10 system, we could estimate incidence rate (IR) of categories of gallstone events (Cholelithiasis, Cholangitis, and Cholecystitis) after 1993, see Table S2 for grouping of diagnosis.

The date of inclusion marked the start of follow-up, and patients and comparators were followed until the first record of a gallstone event, death, emigration, or end of the study, whichever occurred first.

Information on comorbidities related with gallstones was accessed throughout the study period and information on prescription medicine use was accessed through the Danish National Prescription Database (DNPD), starting in 1996. The DNPD contains information on dispensed prescriptions, including variables at the level of the drug user, the prescriber, and the pharmacy [23].

2.3 | Statistics

Sex distribution and median age were determined at inclusion. The number of first-ever gallstone events was counted, and median age with interquartile range (IQR) at the time of the events and time from inclusion to an event were estimated. The cumulative incidence proportion of gallstone events, with death and emigration considered as competing events, were computed for each RBC disorder category and corresponding comparators after 5 years and 10 years of follow-up. The incidence of gallstone events was calculated as IR per 100 000 person-years (PY). IRs, incidence rate ratios, and cause-specific hazard ratios (csHRs) were estimated with 95% confidence intervals (95% CI). Cause-specific HRs were adjusted for age at diagnosis, sex, and hospital diagnoses of diabetes mellitus, dyslipidemia, and obesity. Cause-specific HRs for patients and comparators are depicted in coefficient plots.

2.4 | Sensitivity Analyses

We undertook three sensitivity analyses each excluding part of the outcome define data: (A) Detection of gallstone events only from all discharge diagnoses excluding surgical codes; (B) Events only detected from the primary discharge diagnoses; and (C) outcomes based on surgical procedure codes. All analyses were repeated for each sensitivity analysis. To evaluate the impact of sex on the results, we include a sensitivity analysis stratifying on sex, and repeated the main analyses separate for each sex.

3 | Results

Our study included 9354 patients with congenital RBC disorders and 416 994 comparators. Patients were followed for 79 863 PY, with a median observation time of 6.1 years. Comparators were followed for 4 241 304 PY, with a median observation time of

7.7 years. For nearly all types of RBC disorders, gallstone events occurred markedly more frequently among patients than in the general population. Disease-specific risk estimates are given in Tables 1 and S3 and described below. Disease-specific risk estimates are illustrated in Figure 1. Additional information regarding time to event, risk time, cumulative incidence at 5 years, and risk differences after 5 years and 10 years are provided in Table S5.

3.1 | Hereditary Spherocytosis

HS was the only isolated membrane disorder in our analysis, affecting 1112 individuals. The median age at first gallstone event was 25.0 years [IQR 12.0–53.5 years] in patients with HS versus 47.5 years [IQR 31.9–68.5 years] in the 55 270 comparators. In HS patients, the IR of gallstone events was 892.2 [95% CI 755.1–1054.2] per 100 000 PY and the cumulative incidence at 10 years was 9.8% [95% CI 8.1–11.8]. The adjusted csHR was 8.1 [95% CI 6.8–9.7] for HS patients versus comparators.

3.2 | Enzyme Disorders

For enzyme disorders, our study included 370 patients with G6PD deficiency and 17 283 matched comparators. The age at the first gallstone event was lower in patients with G6PD than in comparators (38.3 years [IQR 6.1–47.7 years] versus 50.0 years [IQR 32.2–77.0 years]). Among patients with G6PD, the IR of gallstone events was 200.7 [95% CI 100.4–401.2] per 100 000 PY and the cumulative incidence at 10 years was 2.5% [95% CI 1.1–4.8]. The adjusted csHR was 3.3 [95% CI 1.6–6.8] for HS patients versus comparators.

In addition, 32 patients with PKD were matched to 1508 comparators. The median age at first gallstone event in patients with PKD was the lowest among all patient groups, at 11.7 years [IQR 7.7–16.6 years] versus 66.7 years [IQR 31.2–87.7 years] in comparators. The IR of gallstone events was 3618 [95% CI 1946-6724] per 100 000 PY in patients with PKD and the cumulative incidence at 10 years was as high as 20.1% [95% CI 8.1%–35.8%]. The adjusted csHR was 21.6 [95% CI 10.6–44.1] for PKD patients versus comparators.

3.3 | Hemoglobin Disorders

For SCD, the IR of gallstone events was 578.7 [95% CI 311.4–1075.5] per 100 000 PY and the cumulative incidence at 10 years was 4.2% [95% CI 1.8–8.1]. When comparing SCD patients to matched patients from the general population, the adjusted csHR of gallstone events was 3.7 [95% CI 1.9–7.0]. Our study also included 625 patients with SCT, 1448 patients with the α-thalassemia trait, 2521 patients with β-thalassemia minor, and 23 325, 60 492, and 114 143 sex-, age-, and region of origin-matched comparators, respectively. In each of these groups, the age at gallstone events was similar among patients and comparators. Within the group of SCT, α-thalassemia trait and β-thalassemia minor patients, the most prominent increased risk of gallstone events was among β-thalassemia minor patients, with an adjusted csHR of 1.8 [95% CI 1.4–2.3]. We also found a slightly higher risk in α-thalassemia trait patients, but no elevated risk in patients with the SCT (Table 1).

3.4 | Other Congenital Red Blood Cell Disorders

We conducted an analysis of 3007 patients in a heterogeneous group comprising patients with other unspecified congenital RBC disorders and 134 734 matched comparators. The median age at first gallstone event was younger in patients with other types of congenital RBC disorders than in comparators (61.7 years [IQR 42.3–76.1 years] versus 69.8 years [IQR 48.4–81.2 years]). The IR of gallstone events was 502.3 [95% CI 419.7–601.2] per 100 000 PY among patients with other congenital RBC disorders, while the cumulative incidence at 10 years was 3.7% [95% CI 3.0%–4.5%]. The adjusted csHR was 2.1 [95% CI 1.8–2.6] comparing other congenital RBC disorder patients with the matched individuals from the general population.

3.5 | Categories of Gallstone Events

For the analysis on categories of gallstone events there were 247 patients with any type of gallstone event and 4814 comparators, with IR of 407.9 [95% CI 360.1–462.1] and 167.0 [95% CI 162.4–171.8], respectively. A person could have been assigned more than one diagnosis code at the same time, partly inflating the numbers when subdivided into categories. Cholelithiasis was registered among 195 patients and among 4.121 comparators, IR 318.0 [95% CI 276.3–365.9]/100 000 PY and 140.9 [95% CI 136.7–145.3]/100 000 PY, respectively. Cholangitis was registered in 9 patients and 118 comparators, IR: 14.1 [95% CI 7.4–27.2]/100 000 PY and 3.9 [95% CI 3.3–4.7], respectively. Cholecystitis was registered among 58 patients and in 959 comparators, IR: 91.7 [95% CI 70.9–118.6]/100 000 PY and 31.9 [95% CI 30.0–34.0]/100 000 PY, respectively.

3.6 | Sensitivity Analyses

We performed various sensitivity analyses. When the csHR was computed based only on surgical procedures, estimates were higher for patients with HS and PKD compared with the main analysis. There were only minor differences from the main analysis in the results of the sensitivity analyses applying only discharge diagnoses excluding surgical codes or primary discharge diagnoses (Table S3). Stratification on sex did not change the estimates and restricted analyses had nonsignificant deviations, mainly due to smaller analysis population. When only including women the adjusted csHR among patients with PKD or G6PD deficiency decreased to 12.7 [95% CI 4.4–37.0] and 1.7 [95% CI 0.4–6.9], respectively. For men the adjusted csHR among patients with PKD, G6PD deficiency, SCD, or α-Thalassemia trait increased to: 41.0 [95% CI 14.5–116.0], 5.2 [95% CI 2.3–12.0], 12.0 [95% CI 4.3–33.7], and 2.4 [95% CI 1.0–6.0], respectively.

4 | Discussion

The risk of a hospital diagnosis of gallstone disease in patients with congenital RBC disorders was found to be considerably

TABLE 1 | Gallstones in patients with congenital red blood cell disorders, compared with matched individuals from the general population, Denmark, 1980–2016.

Diagnosis	Number of patients	Median age at gallstone event (years)	Incidence rate (/100 000 PY)	Adjusted csHR[a]	Cumulative incidence 10 years (%)
HS	1122	25.0 IQR 12.0–53.5	892.2 [755.1–1054.2]	8.1 [6.8–9.7]	9.8 [8.1–11.8]
G6PD	370	38.3 IQR 6.1–47.7	200.7 [100.4–401.2]	3.3 [1.6–6.8]	2.5 [1.1–4.8]
PKD	32	11.7 IQR 7.7–16.6	3618.1 [1946.7-6724.4]	21.6 [10.6–44.1]	20.1 [8.1–35.8]
SCD	229	31.4 IQR 18.3–39.5	578.7 [311.4–1075.5]	3.7 [1.9–7.0]	4.2 [1.8–8.1]
SCT	625	41.1 IQR 37.5–45.8	226.0 [117.6–434.3]	0.8 [0.4–1.6]	1.2 [0.5–2.5]
α-Thalassemia trait	1448	41.1 IQR 29.1–46.8	342.0 [244.3–478.6]	1.5 [1.1–2.2]	3.3 [2.2–4.7]
β-Thalassemia minor	2521	44.3 IQR 34.8–55.7	341.5 [270.7–431.0]	1.8 [1.4–2.3]	3.1 [2.3–4.0]
Other congenital hemolysis	3007	61.7 IQR 42.3–76.1	502.3 [419.7–601.2]	2.1 [1.8–2.6]	3.7 [3.0–4.5]

Note: Values in square brackets indicate 95% confidence interval.

Abbreviations: csHR, cause-specific hazard ratio; G6PD, glucose-6-phosphate dehydrogenase deficiency; HS, hereditary spherocytosis; IQR, interquartile range; PKD, pyruvate kinase deficiency; SCD, sickle cell disease; SCT, sickle cell trait.

[a]Cause-specific hazard ratios are adjusted for age at diagnosis, sex, hospital-diagnosed diabetes mellitus, dyslipidemia, and obesity.

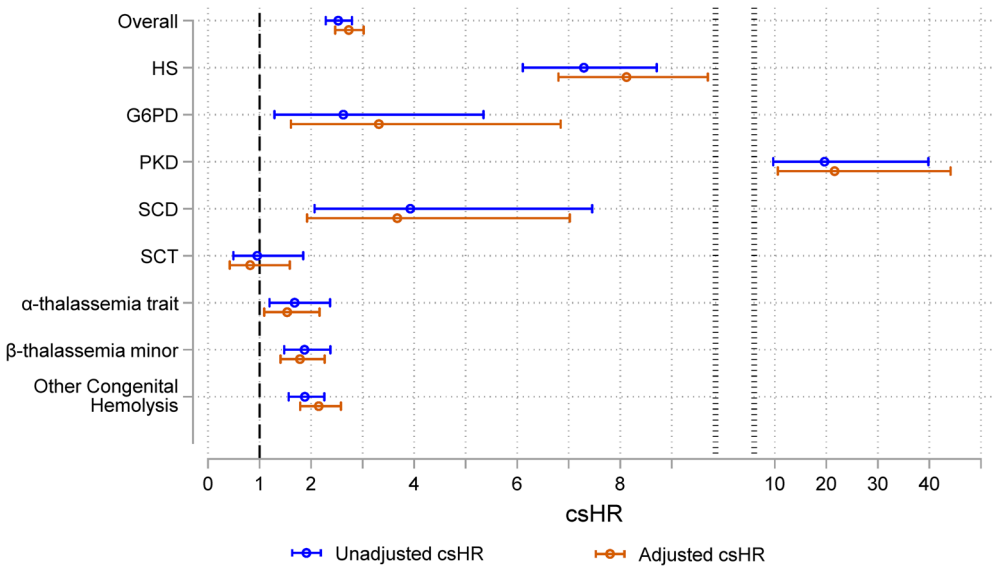

FIGURE 1 | Cause-specific hazard ratios of gallstones in patients with congenital red blood cell disorders, compared with matched individuals from the general population. Whiskers indicate 95% confidence intervals around estimates. The *x*-axis has a shift in scale because of higher values for pyruvate kinase deficiency. csHR values are adjusted for age at diagnosis, sex, diabetes mellitus, dyslipidemia, and obesity. G6PD, glucose-6-phosphate dehydrogenase deficiency; HS, hereditary spherocytosis; PKD, pyruvate kinase deficiency; SCD, sickle cell disease; SCT, sickle cell trait. [Color figure can be viewed at wileyonlinelibrary.com]

elevated and to occur at an earlier age than in matched comparators from the general population. To the best of our knowledge, this excess risk has not been previously reported. Our findings indicate that the risk of hospital-diagnosed gallstone events was between 2 and 22 times higher among almost all groups of individuals with congenital RBC disorders than in the general population. Only patients with the SCT did not demonstrate a higher risk. The risks were generally 1.5–2.5 times higher in all patients with congenital RBC disorders, but patients with HS and PKD had remarkably higher risks.

4.1 | Interpretation

Prior studies have reported an ultrasound-ascertained gallstone prevalence of 31%–41% in children and young adults with HS [13, 14]. Although not directly comparable to the general population, this prevalence is likely markedly higher than the overall low prevalence of gallstones—<2%—reported in children [24, 25]. Accordingly, our finding of an 8.1-fold elevated risk of gallstone disease and earlier diagnosis in patients with HS is in line with these findings. In patients with PKD, we found a csHR of 21.6 compared with the general population and a 10-year cumulative incidence above 20%. Although prior studies on this topic are sparse, Grace et al. [16] reported a gallstone prevalence of 45% in a mixed cohort of children and adults. Our findings underscore that patients with PKD not only are at high risk of developing gallstones but also have an increased likelihood of experiencing symptomatic disease, evidenced by their higher rate of hospital-diagnosed gallstones.

Although the results of our study and that of Grace et al. both showed large increases in the same direction, their combined findings are based on only 280 patients; Thus, further confirmation of the increased risk will be required in other cohorts. As for G6PD deficiency and gallstone disease Kılıç et al. [15] reported an overall gallstone prevalence of 4.8% in children with G6PD younger than 18 years. Our estimate of a 3.3-fold higher risk of symptomatic gallstone disease among patients with versus without G6PD deficiency corroborates that gallstone prevalence can manifest in overt clinical problems.

Among congenital RBC disorders, the association between SCD and gallstones has been the most extensively examined. A recent meta-analysis estimated a combined gallstone prevalence of 25.3% in adults and children, with a prevalence of 44.1% in adults with diagnosed with SCD [6]. Although most studies included in the meta-analysis did not account for symptoms, the high prevalence suggests an elevated risk of symptomatic gallstones. Our results align with this possibility: we estimated an adjusted csHR of 3.7 for gallstones in patients with SCD compared with the general population. The prevalence of gallstones or gallstone-related symptoms in persons with SCT is unknown. As expected, our results do not indicate elevated gallstone risk, because affected persons rarely demonstrate clinically relevant chronic hemolysis. Similarly, persons with the α-thalassemia trait often exhibit no or only mild anemia and only slightly elevated hemolytic activity. Consequently, we found an only small increase in gallstone risk, potentially reflecting random variation or uncontrolled confounding. Prior results directly comparable to ours were available for only β-thalassemia minor. An odds ratio of 2.0 has been reported for women with β-thalassemia minor compared with a matched group of women without this condition [7]. In our cohort, the adjusted csHR for gallstone events was 1.8, indicating that β-thalassemia minor, despite causing few symptoms, is a risk factor for gallstones in both men and women of all ages after adjustment for other common risk factors. Most studies on the association between gallstones and hemoglobin disorders have focused on transfusion-dependent β-thalassemia, whose reported prevalence ranges from 30.5% to 100% in different populations [8, 9]. Because these diseases are rare in ethnic Danes and thereby in our cohort, we combined

them with other rare diseases, thus yielding an adjusted csHR of 2.1, not readily attributable to transfusion-dependent thalassemia. Instead, these findings emphasize the presumed underlying association between chronic hemolysis of any type and elevated risk of gallstone disease.

Based on the information available, the chemical composition of gallstones in this cohort couldn't be determined. As mentioned in the introduction, chronic hemolysis potentially favors the formation of black pigment gallstones. However, RBC disorder patients are also at risk of classic cholesterol stones, which are the most common in the general population. We have adjusted for known risk factors of cholesterol stones in our analyses and presume on that basis, that the elevated risk of gallstones in RBC disorder patients, primarily relates to black pigment gallstone.

4.2 | Limitations

Our study was based on a large population of patients with congenital RBC disorders and matched comparators, using nationwide data with near complete inclusion of patients and complete follow-up. Because our data originate from public hospital records, we cannot exclude the possibility that a few patients might have been treated for gallstone-related diseases at private hospitals, thus decreasing data capture of associated surgeries. Consequently, our rate estimates might be conservative. However, because any incomplete capture was likely to apply equally to patients and the general population, the relative results might not have been affected substantially. In addition, we expect that most private hospital surgeries would be preceded by a prior diagnosis of gallstone disease at a public hospital, and consequently recorded in the DNPR. This is supported by our sensitivity analyses, which indicated that exclusion of surgical procedures had only a small effect. As diagnostic and surgery codes are provided when a disease results in current hospitalization, we used the ICD-8, ICD-10, and surgery codes pertaining to gallstones, as marker of symptomatic gallstone disease. However, a possible study limitation is that we cannot exclude the possibility that patients could have been assigned a gallstone-related diagnosis code with no gallstone symptoms. However, when we restricted the analysis to the primary diagnosis leading to hospital contact, to minimize the effects of such potential surveillance bias, only minor changes occurred in the overall results. We also include genetic RBC disorder carriers, as α-thalassemia trait, β-thalassemia trait and SCT, which in most cases are diagnosed and followed in general practice. We find a similar overall signal in these persons, SCT being the exception, even though these persons are less likely to be subjected to a surveillance bias. When we estimated csHR based only on surgical procedures, the estimates were higher for patients with HS or PKD. This finding might indicate a tendency to perform concomitant cholecystectomy and splenectomy among patients with HS or with a lesser indication in both patient groups.

4.3 | External Validity

The results presented in this study are based on a nationwide cohort of all patients with congenital RBC disorder in Denmark (from 1980 to 2016), with no exclusions, thus increasing the external validity of our results. Still, some of the investigated diseases are so rare that confirmation using other cohorts is needed, especially for PKD.

5 | Conclusion

We found a markedly higher risk of hospital-diagnosed gallstone disease in almost all groups of patients with congenital RBC disorders compared with the general population, based on nationwide data.

Ethics Statement

The project was approved by the Southern Danish Region record no. 17/10885.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

According to Danish law the authors cannot grant direct access to the data from the national health registers. Researchers may apply for use of research data to Statistics Denmark.

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Supporting Information

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