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The German sickle cell disease registry reveals a surprising risk of acute splenic sequestration and an increased transfusion requirement in patients with compound heterozygous sickle cell disease HbS/β-thalassaemia and no or low HbA expression

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Abstract

Patients with sickle cell disease (SCD) in Germany exhibit a substantial genetic diversity in the β-globin genotype. Data collected by the national German SCD registry reflect this diversity and allowed us to analyze the phenotypes associated with different SCD genotypes. Our study focused on 90 patients with HbS/β-thalassaemia (HbS/β-thal) and compared these to patients with HbSS and HbSC. Patients with HbS/β-thal were classified into three groups: HbS/β^0-thal (no HbA), HbS/β^+-thal (HbA < 14%), and HbS/β^{++}-thal (HbA≥14%). In comparison to HbSS, patients with HbS/β^{++}-thal had higher Hb-levels, lower hemolytic activity and rarely required red blood cell transfusions. HbS/β^0-thal and HbS/β^+-thal closely resembled each other and are jointly referred to as HbS/$\beta^{0/+}$-thal. Compared to HbSS, patients with HbS/$\beta^{0/+}$-thal experienced a similar frequency of vasoocclusive crises and degree of

A full list of the members of the Sickle Cell Disease Study Group appears in the Supplementary Appendix.

hemolysis. However, the frequency of red blood cell transfusions (0.6 vs. 0.39/year, $p = .0049$) and splenic sequestration crises (42.4 vs. 15.5% of patients, $p = 3.799e\text{-}05$) was higher in HbS/$\beta^{0/+}$-thal than in HbSS, but close to zero in HbS/β^{++}-thal. In conclusion, the level of HbA expression determines the phenotype of HbS/β^{+}-thal. HbS/β-thal expressing no or little HbA is hematologically similar to HbSS, but causes a previously unknown high risk of splenic sequestration.

KEYWORDS
anemia, sickle cell, thalassemia

1 | INTRODUCTION

Compared to countries where sickle cell disease (SCD) is endemic or has been prevalent for centuries as a result of colonial history, most patients with SCD in Germany have immigrated in recent years.[1] According to a recent estimate, 3200 patients with SCD live in Germany.[2] These patients come from many different regions, including sub-Saharan Africa, the Mediterranean region, and the Middle East.[1,3] Due to the ethnic heterogeneity of patients in Germany, the genotypes of SCD are diverse. Besides homozygous HbSS, compound heterozygous SCD (HbSC and HbS/β-thal) account for approximately one quarter of all patients with SCD in Germany.[3]

A nationwide registry of patients with SCD began collecting data on disease characteristics, complications, and treatment in 2015 and currently covers approximately one third of all patients in Germany. Most patients in the registry are treated at specialized institutions and receive care according to national guidelines[3] (see Supplementary Methods). The ethnic diversity of the patient population, the frequent use of hydroxyurea, and the nationwide patient registry allow for the identification of genetic traits that influence the frequency of complications in patients with SCD under current conditions.[4]

The *HBB* genotype is considered the main modifier of the SCD phenotype. While homozygous SCD and HbSC are homogenous with respect to the *HBB* coding sequence, HbS/β-thal is characterized by a heterogeneous variety of more than 300 different thalassemic *HBB* variants. HbS/β-thal is classified as HbS/β^{0}-thal if no physiological β-globin is expressed, and HbA cannot be detected. HbS/β^{0}-thal and HbSS are frequently considered clinically indistinguishable, and both commonly referred to as "sickle cell anemia",[5–8] in contrast to the other forms of compound heterozygous SCD. Here, we focus on the effects of the *HBB* genotype on the phenotype of HbS/β-thal.

Patients whose thalassemic *HBB* allele allows for residual expression of normal β-globin (HbS/β^{+}-thal) are considered to have a milder course of the disease because HbA can interfere with HbS polymerization. As a consequence, treatment guidelines do not generally recommend prophylactic measures for patients with HbS/β^{+}-thal that are routine for HbSS, such as penicillin prophylaxis and screening with transcranial Doppler ultrasound.[5,9,10]

Acute splenic sequestration crisis (ASSC) belongs to the early life-threatening complications from SCD and often first happens before the age of 5.[11,12] ASSC mainly affects young children with HbSS but also older patients with HbS/β-thal,[13–15] likely because repeated infarctions occurring in HbSS typically result in autosplenectomy during infancy or early childhood,[6,16] while the higher levels of HbA and/or HbF in HbS/β-thal preserve splenic function until an older age.[12,17] The frequency of ASSC in HbS/β-thal was reported to be similar[14,18,19] or higher[20–22] compared to HbSS.

The minimal concentration of HbA required to efficiently slow down HbS polymerization and thus ameliorate hemolysis and vasoocclusion in HbS/β-thal is unknown. Nevertheless, HbA expression was previously used to classify HbS/β^{+}-thal: In type 1, HbA represents less than 7% of the total hemoglobin, in type 2 HbA levels are between 7 and 14% and in type 3 HbA is >14%.[23,24] Based on previous reports,[18,23,25,26] we hypothesized that the residual expression of HbA in HbS/β^{+}-thal type 1 and 2 may not be sufficient to improve the course of SCD. The question if patients with HbS/β-thal and an *HBB* allele that allows for minimal HbA expression clinically differ from patients with HbSS and HbS/β^{0}-thal has not been conclusively answered but is important for the management of these patients.

2 | METHODS

2.1 | Patient recruitment & data collection

Patients were enrolled in the study through the nationwide German SCD registry (NCT03327428), which collects prospective and retrospective data on patients with SCD in Germany.[3] The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board of the Medical Faculty of Heidelberg University (S-416/2014). Patients or legal guardians provided written informed consent. Data were collected from November 2015 and include demographic information, genotype, treatment, laboratory parameters and clinical events. The data were locked on April 15th, 2022 and included 624 patients with homozygous SCD and 186 patients with compound heterozygous SCD from 31 different institutions (Table S9). Patients with SCD whose genotype was not specified (missing data), patient data collected after stem cell transplantation and during chronic transfusion were excluded. Four patients with a genotype other than HbSS, HbSC, or HbS/β-thal were also excluded from the analysis (HbS/D Punjab ($n = 2$), HbS/HPFH, HbS/OArab). All co-authors had access to all registry data.

For the analysis of laboratory parameters and complications of SCD, only patients aged 2 years or older were included. In the analysis

of acute splenic sequestration crisis (ASSC), infants younger than 2 years were included in addition. Complications such as vasoocclusive crises (VOC), stroke, sepsis, or ASSC were assessed using retrospectively and prospectively collected data (see Supplementary Methods for definitions). To minimize recall bias, retrospective data that referred to the period before enrollment into the registry were only considered if the age at registration was below 18 years. Data on complications and treatment of SCD, including transfusions, were extracted by chart review annually and documented by study nurses and documentalists in the participating study centers, along with laboratory parameters (Hb, MCV, reticulocytes, LDH, bilirubin, and HbF) that were preferentially taken during routine checkups, not during complications of SCD. The latter were only considered if the patient had not received red blood cell transfusions within 100 days before laboratory assessment. If more than one laboratory data point per patient met these criteria, the average of the available data was used.

2.2 | Classification of HbS/β^{+}-thal

We used Serjeant's classification which categorizes HbS/β^{+}-thal into three types based on HbA levels: type 1 (1%–7% HbA), type 2 (7%–14% HbA), and type 3 (14%–25%).[23] Using this definition, we classified patients according to the HbA level associated with their respective thalassemic variant using publicly available databases (HbVar,[27] IthaNet,[28] see Supplementary Methods). Patients with either a type 1 or type 2 thalassemic variant were grouped together as HbS/$\beta^{0/+}$-thal. Patients with a type 3 thalassemic variant were defined as HbS/β^{++}-thal.

2.3 | Statistical methods

Differences in hematological parameters and the frequency of complications between the genotypes of SCD were evaluated using the Kruskal-Wallis test, with pairwise comparisons assessed using the Wilcoxon rank sum test. Holm correction was used to adjust the p-values for multiple comparisons. To investigate differences between the genotypes in terms of the risk for splenic sequestration, stroke, sepsis, and splenectomy, logistic regression was used, taking into account the different observation durations for patients. All statistical analyses were conducted using R version 4.2.1 (The R Foundation for Statistical Computing 2022), and $p < .05$ was considered significant.

3 | RESULTS

3.1 | Patients' characteristics

We identified 90 patients with compound heterozygous SCD HbS/β-thal in the registry and compared them to 624 patients with HbSS and 92 patients with HbSC (Table 1). The distribution of sex and age did not differ between the genotypes. Patients with HbS/β-thal originated predominantly from the Mediterranean region, patients with HbSS and HbSC from sub-Saharan Africa.

With IVS-I-110 G > A being the predominant thalassemia variant, most patients with HbS/β-thal had genotypes that express little (<14%) or no HbA. Approximately 20% of patients with HbS/β-thal carried a type 3 β-thalassemia variant with HbA expression exceeding 14% of the total hemoglobin (Table 2).

As expected, patients with HbS/β^{+}-thal and a type 3 thalassemic variant had higher total hemoglobin levels, lower HbF, lower reticulocyte count, lower LDH and lower ferritin compared to patients with a type 1 and type 2 variant (see Table S1).

3.2 | HbS/β^{0}-thal and HbS/β^{+}-thal type 1 and 2 are very similar

To test if the residual expression of HbA in HbS/β^{+}-thal type 1 and 2 is sufficient to improve the phenotype of SCD, we compared laboratory parameters and frequency of complication of patients with HbS/β^{0}-thal to patients with HbS/β^{+}-thal type 1 and 2 (see Table S2). We found that patients with HbS/β^{+}-thal type 1 and 2 showed no

TABLE 1 Patients' characteristics.

		HbS/β^{+}-thal	HbS/β^{0}-thal	HbSS	HbSC
Sex	Female	29 (54%)	18 (50%)	304 (49%)	42 (46%)
	Male	25 (46%)	18 (50%)	320 (51%)	50 (54%)
Age (years)	*n*	50	34	548	85
	Mean + SD	15.6 ± 9.9	12.3 ± 8.4	12.7 ± 8.8	13.4 ± 8.4
	Range	2.9–50.5	3.1–51.4	2–57.2	2.5–47.3
Origin[a]	SSA	12 (24%)	3 (8%)	425 (68%)	77 (95%)
	MR	22 (45%)	25 (69%)	77 (24%)	3 (4%)
	Others	12 (24%)	6 (17%)	38 (6%)	1 (1%)
	Mixed	3 (6%)	2 (6%)	8 (2%)	0

Note: *n* refers to the number of evaluable patient and may differ between categories because of missing data. SD: standard deviation. Details on a subgroup of patients with HbSS were published previously.[4]
[a]Region of origin: SSA: Sub-Saharan Africa; MR: Mediterranean Region; others: Iraq $n = 23$, all others $n \leq 3$ each; Mixed: parents from different region of origins.

TABLE 2 Thalassemic variants in patients with HbS/β-thal.

HbS/β^0-thal $n = 36$		HbS/β^+-thal type 1, $n = 7$		HbS/β^+-thal type 2, $n = 22$		HbS/β^+-thal type 3, $n = 17$	
Variant	***n***	**Variant**	***n***	**Variant**	***n***	**Variant**	***n***
IVS-I-1 G > A	7	IVS-I-5 G > C	5	IVS-I-110 G > A	22	−29 (A > G)	4
IVS-II-1 G > A	4	IVS-I-5 G > A	2			IVS-I-6 T > C	4
IVS-II-849 A > C	2					−88 (C > T)	4
IVS-II-849 A > G	1					−88 (C > A)	3
CD 5 (-CT)	2					IVS-II-848 C > A	1
CD 8 (−AA)	2					PolyA (T > C)	1
CD 15 TGG > TAG	1						
CD 36/37 (−T)	1						
CD 39 CAG > TAG	7						
CD 41/42 (-TTCT)	1						
Not specified[a]	8						

[a]Eight patients were reported as HbS/β^0-thal but neither the β-globin mutation analysis nor HbA levels were available. These patients were excluded from further analyses.

differences in total hemoglobin, HbS, mean corpuscular volume (MCV), leucocytes, platelets, reticulocytes, ferritin, bilirubin, and lactate dehydrogenase (LDH) compared to patients with HbS/β^0-thal. The mean fetal hemoglobin (HbF) level in patients with HbS/β^0-thal was slightly higher than in patients with HbS/β^+-thal type 1 and 2, suggesting that γ-globin expression with subsequent HbF increase partially compensated the missing β-globin expression in HbS/β^0-thal. However, the higher HbF levels in HbS/β^0-thal did not translate into clinical advantage as the frequencies of VOC, acute chest syndrome (ACS), and red blood cell transfusions did not differ between both groups. Therefore, we chose to consider HbS/β^+-thal type 1 and 2 and HbS/β^0-thal as one group, referred to as HbS/$\beta^{0/+}$-thal, and compared this group to patients with HbS/β^+-thal type 3 with HbA >14% of the total hemoglobin, referred to as HbS/β^{++}-thal (see Figure 1; Table S3).

3.3 | HbS/$\beta^{0/+}$-thal is associated with frequent acute splenic sequestration crises and transfusions

To identify groups of patients who are particularly at risk for splenic sequestration crisis (ASSC), we analyzed the proportion of patients aged over 2 who had either a documented ASSC in their medical history or had experienced an ASSC during follow-up in the registry (Table 3). Overall, out of 662 evaluable patients of all genotypes, 107 (16%) had ever experienced an ASSC. This proportion was almost three times higher in patients with HbS/$\beta^{0/+}$-thal than in those with HbSS ($p = 3.799e\text{-}05$). Most ASSC occurred before registration (retrospective: 91, vs. prospective after registration: 30 ASSC), thus few data on the age at the event are available (Table S7). In ASSCs that were prospectively documented in the registry, hemoglobin levels and the age at time of ASSC did not differ significantly between HbS/$\beta^{0/+}$-thal and HbSS (Table S7; $p = .26$). Likely as a consequence of ASSC, the proportion of patients who had undergone splenectomy was highest in HbS/$\beta^{0/+}$-thal (35%, compared to 13% in HbSS, $p < 10e\text{-}4$). Most splenectomies were reported as part of the patient's history before enrollment in the registry and details on indication and circumstances of splenectomy are not available.

Patients with HbS/$\beta^{0/+}$-thal also had the highest transfusion requirements of all genotypes (0.61/year compared to 0.36/year in HbSS, $p = .035$, Figure 1; Table S3). We hypothesized that the higher transfusion requirement may be a consequence of more frequent ASSC and thus compared the frequency of transfusion after excluding all patient years with at least one ASSC reported. However, the observed difference in transfusion requirements was still present after excluding all years with ASSC (HbS/$\beta^{0/+}$-thal 0.58/year vs HbSS 0.35, $p = .0418$; Table S4), indicating that ASSC is not the only reason for the high transfusion requirement in patients with HbS/$\beta^{0/+}$-thal. This finding was corroborated by the fact that the transfusion requirement in patients with HbS/$\beta^{0/+}$-thal who had undergone splenectomy was not reduced in comparison to those without splenectomy (Table S5).

3.4 | HbS/β^{++}-thal is characterized by low hemolytic activity

While HbSS and HbS/$\beta^{0/+}$-thal hardly differ hematologically, HbS/β^{++}-thal is characterized by higher total hemoglobin, lower HbF and lower hemolytic activity (reticulocytes, bilirubin, and LDH; Figure 1; Table S3). None of the patients with HbS/β^{++}-thal had a history of ASSC. Importantly, patients with HbS/β^{++}-thal did not require any red blood cell transfusions while being followed in our registry (47 patient years; Wilcoxon pairwise comparison to HbSS $p = .028$ and to HbS/$\beta^{0/+}$-thal $p = .0058$). Serum ferritin, which is a marker of transfusion history and chronic inflammation, was significantly lower in HbS/β^{++}-thal compared to the other genotypes, except HbSC. However, the frequencies of VOC and ACS in HbS/β^{++}-thal were not significantly different from those in HbSS and HbS/$\beta^{0/+}$-thal.

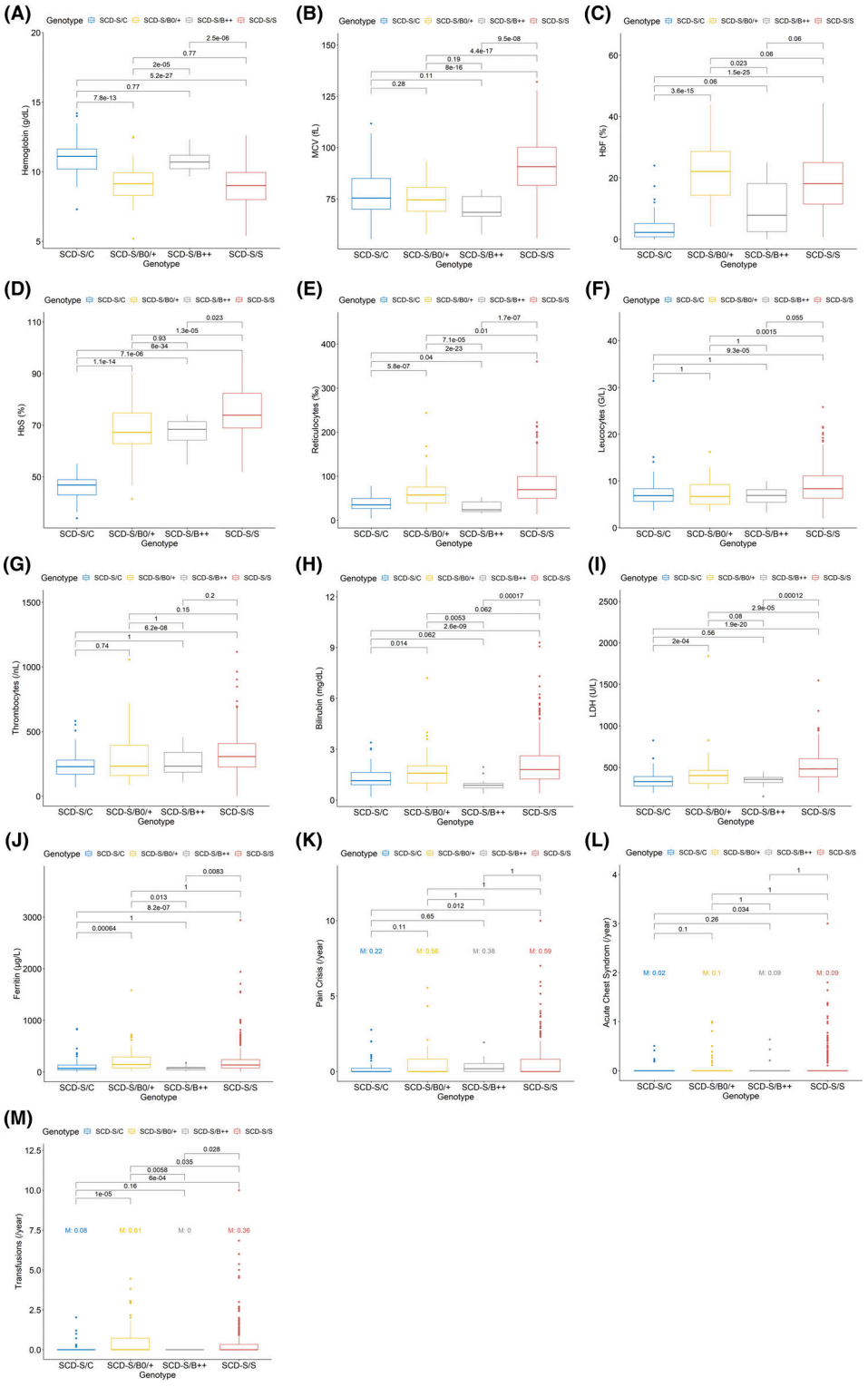

FIGURE 1 Hematological parameters and frequency of complications in HbSC, HbS/β0/+-thal, HbS/β++-thal, and HbSS. (A) Total hemoglobin; (B) MCV; (C) Hb F; (D) Hb S; (E) Reticulocytes; (F) Leucocytes; (G) Thrombocytes; (H) Total Bilirubin; (I) LDH; (J) Ferritin; (K) frequency of pain crises; (L) frequency of acute chest syndrome; (M) Frequency of red blood cell transfusions. (K–M) mean values are annotated over boxplots. Post-hoc Wilcoxon test was conducted with Bonferroni-Holm correction for pairwise comparisons (p values over brackets). $p < .05$ was considered significant.

3.5 | Treatment of SCD in Germany

Similar to homozygous HbSS, hydroxyurea is used in >90% of patients with HbS/$\beta^{0/+}$-thal in registry patients who are 2 years or older (Table 4). In contrast, approximately 60% of patients with HbS/β^{++}-thal were prescribed hydroxyurea, similar to patients with HbSC. The mean age at initiation of hydroxyurea and the mean dose was similar for all patients, consistent with the recommendations of the German treatment guidelines for SCD.[29] The higher use of hydroxyurea in HbS/$\beta^{0/+}$-thal may have contributed to higher HbF levels compared to HbS/β^{++}-thal (Tables S1 and S2).

TABLE 3 Proportion of patients with a history of acute splenic sequestration crisis, splenectomy, sepsis and stroke by SCD genotype.

		HbS/$\beta^{0/+}$-thal	HbS/β^{++}-thal	HbSS	HbSC
Acute splenic sequestration crisis	Yes	25 (43.1%)	0 (0%)	80 (15.6%)	2 (2.6%)
	n	*58*	*13*	*513*	*78*
	p[a]	**3.799e-05**	1.028e-01	/	**1.034e-04**
Splenectomy	Yes	21 (35%)	1 (7.7%)	72 (13%)	2 (2.5%)
	n	*60*	*13*	*553*	*79*
	p[a]	**2.71e-04**	.502	/	**.009**
Sepsis	Yes	2 (3.6%)	2 (14.3%)	35 (6.1%)	1 (1.3%)
	n	*56*	*14*	*575*	*77*
	p[a]	.454	.325	/	.113
Stroke	Yes	2 (3.3%)	1 (7.1%)	24 (4.2%)	0 (0%)
	n	*60*	*14*	*578*	*79*
	p[a]	.819	.449	/	**.043**
TCD results	≥200 cm/s	3 (6.4%)	0	26 (6%)	0
	<200 cm/s	44	10	404	55
	n	47	10	430	55

Note: *n* refers to the number of evaluable patients for each parameter. $p < .05$ (in **bold**) was considered significant.
Abbreviation: SCD, sickle cell disease.
[a]Logistic regression compared to HbSS.

TABLE 4 Care for patients with SCD.

	HbS/$\beta^{0/+}$-thal	HbS/β^{++}-thal	HbSS	HbSC
Mean observation time in the registry (years)	2.7 ± 1.7	3.2 ± 1.4	2.8 ± 1.6	2.8 ± 1.7
Hydroxyurea treatment[a]	58 (96.7%)	9 (60%)	518 (93.2%)	51 (60.7%)
Hydroxyurea daily dose (mg/kg)	20.9 ± 6.8	18.9 ± 7.6	22.2 ± 6.1	18.1 ± 5.7
Mean age at start of Hydroxyurea (years)	8 ± 7.2	10.8 ± 8.6	7 ± 6.1	10.5 ± 6.9
Chronic transfusions	4 (7%)[b]	0	51 (9.9%)	0
TCD-screening (during recent 2 years)[c]	36 (58.1%)	9 (60%)	382 (68.7%)	41 (47.1%)

Note: Only patients with available data were counted.
Abbreviation: SCD, sickle cell disease.
[a]In patients >2 years.
[b]Indications: Priapism ($n = 1$), chronic pain ($n = 1$), symptomatic anemia ($n = 1$), repeated severe VOC ($n = 1$).
[c]TCD screening refers to patients between the age of 2 and 18 years. German national SCD guidelines do not recommend TCD screening for patients with HbSC.

4 | DISCUSSION

The pathogenic potential of a thalassemic variant in compound heterozygosity with the HbS-variant is considered to be determined by the residual expression of β-globin. Consequently, we grouped patients with HbS/β^{+}-thal by HbA levels according to the classification proposed by Serjeant[23] and observed that hemolysis inversely correlates with HbA expression. Based on our data that integrate genetic, hematologic and clinical parameters, we propose that patients with HbS/β-thal are classified into the HbS/$\beta^{0/+}$-thal phenotype that resembles HbSS and into the HbS/β^{++}-thal phenotype that is associated with less severe anemia. This classification requires either a genetic diagnosis or a quantification of the HbA expression. Although similar classifications have been employed previously,[25,26] we suggest that this classification is validated in further studies that consider additional end points such as chronic complications of SCD and mortality. A precise classification can guide genetic counseling and inform clinical management.[24] In addition, this classification enables the detection of characteristics associated with each phenotype and will be helpful in comparing clinical characteristics of patients with HbS/β-thal between genetically diverse populations. Most patients with HbS/β^{+}-thal in Germany carry thalassemic variants that allow for a minimal expression of HbA only. The most prevalent single thalassemic variant was the IVS-I-110 G > A variant which is common in patients originating from the Mediterranean region (Turkey, Greece, Lebanon, Italy).[18,26,30,31]

The spectrum of thalassemic variants in patients with HbS/β^{+}-thal in our registry is similar to that in Italy,[26] but it differs from

patients of predominantly West-African origin. In the USA,[32] Jamaica,[33] and Guadeloupe[34] most patients with HbS/β^+-thal carry alleles that result in high HbA (>14%), such as the promoter variants −29 A > G and − 88 C > T or the 3'end mRNA processing variant PolyA T > C. In contrast, the most frequent variant in Germany and Italy, IVS-I-110 G > A, results in a residual HbA expression of <14% and has previously been associated with a phenotype that clinically resembles that of HbS/β^0-thal.[25,26] Approximately one third of all patients with SCD in Germany are documented in our registry.[2] While we cannot exclude that clinical characteristics of patients who are not registered may differ from those of registry patients, independent data sources[1,2] imply that the countries of origin of registry patients are representative for all patients with SCD in Germany.[3]

Based on the relatively benign course of patients with HbS/β^+-thal, the NLHBI treatment guidelines[5] recommend withholding screening for the risk of stroke with transcranial Doppler ultrasound and penicillin prophylaxis for all patients with HbS/β^+-thal. According to the predominance of thalassemic alleles with low HbA expression among patients with HbS/β^+-thal confirmed in this study, German treatment guidelines recommend both prophylactic measures, TCD screening and penicillin prophylaxis, for all patients with HbSS and HbS/β^+-thal. Our findings show that the clinical course of most patients with HbS/β^+-thal in Germany, particularly those with low HbA, cannot be distinguished from HbS/β^0-thal or HbSS. The rate of VOC was not significantly different between these genotypes, even including HbS/β^{++}-thal, justifying the use of hydroxyurea in patients with HbS/β-thal.

Although the number of patients who suffered from sepsis is too low to detect possible differences in the frequency of this devastating complication, patients of all genotypes, including HbS/β^{++}-thal and HbSC, presented with sepsis. While we do not know details on these complications, because most were reported retrospectively as part of the patient's history, these data confirm previous observations[25] and justify offering penicillin prophylaxis to all patients with SCD irrespective of the genotype. The clinical course of HbSS is commonly considered indistinguishable from HbS/β^0-thal[6,7,14,23] and treatment guidelines[5,29,35] agree that patients with HbS/β^0-thal should be considered the same in terms of prophylaxis and treatment as patients with HbSS. We suggest that this recommendation be extended to all HbS/$\beta^{0/+}$-thal patients.

In accordance with the ASH recommendations,[9] TCD screening is recommended in Germany for primary stroke prevention in HbSS, HbS/β^0-thal and HbS/β^+-thal, but not in HbSC. Our observations support these recommendations. The proportion of patients with pathological TCD imaging and consequently an indication for chronic red blood cell transfusions is the same in HbS/$\beta^{0/+}$-thal and HbSS. In contrast, we did not observe a pathological TCD result or stroke in HbS/β^{++}-thal or HbSC. Because of the young age and the frequent use of hydroxyurea in our patients, we cannot compare the frequency of TCD abnormalities and stroke to that in historic populations.[36,37]

The incidence of ASSC in patients with HbSS was consistent with previous reports,[11,21] while only very few patients with HbS/β^{++}-thal and HbSC were affected.[21,38] Surprisingly, the proportion of patients with ASSC was almost three times higher in patients with HbS/$\beta^{0/+}$-thal than in those with HbSS. We performed a systematic literature search (Table S8) and identified seven original publications comparing the frequency of ASSC in different SCD genotypes.[14,18–22,25] However, various classifications of the SCD genotypes were employed. We assume that studies combining all genotypes of HbS/β^+-thal[14,18–20,22] were flawed by not discriminating patients with high (HbS/β^+-thal type 1 and 2) and low (HbS/β^+-thal type 3) rates of ASSC, obscuring the difference between HbS/β^{++}-thal and HbS/$\beta^{0/+}$-thal we observed here. In addition, cohorts characterized earlier were often ethnically homogeneous[18,20,21] where the predominance of thalassemic variants with either low or high residual HbA production precluded the detection of possible differences in the frequency of complications. Two publications employed a similar classification of SCD as we did.[21] One detected a strong difference in the frequency of transfusions and ASSC between HbS/$\beta^{0/+}$-thal and HbS/β^+-thal,[25] very alike to our findings. Unfortunately, no comparison to the corresponding numbers in HbSS was provided. Another did not provide genotypic data but classified HbS/β-thal according to HbA expression and found ASSC to be more frequent in HbS/β^0-thal and HbS/β^+-thal, presumably mostly with low HbA expression, than in HbSS.[21] No comparison between HbS/β^0-thal and HbS/β^{++}-thal was provided due to the rarity of β^{++}-thal phenotypes in this population.

The delayed loss of splenic function in HbS/$\beta^{0/+}$-thal may explain the higher proportion of patients who experienced ASSC, as does the extended period of risk due to the frequent use of hydroxyurea in this and previously reported cohort.[39] The age at initiation of hydroxyurea is similar between the different genotypes (Table 4) and cannot explain the difference in the frequency of ASSC. Because most ASSC in our study occurred before enrollment in the registry, our data do not enable to analyze differences in the age when the first ASSC occurred in each phenotype. As current national treatment guidelines recommend parent education on ASSC independent of the SCD genotype, it appears unlikely that the differences in frequency of SCD between HbSS, HbS/$\beta^{0/+}$-thal and HbS/β^{++}-thal are related to differences in the awareness of parents and physicians.

The frequency of red blood cell transfusions in patients with HbS/$\beta^{0/+}$-thal far exceeded that of all other genotypes, including HbSS. This higher need for red blood cell transfusions may be related to splenic sequestration and hypersplenism, but the difference remained significant even after censoring all observation periods with ASSC. Patients with HbS/$\beta^{0/+}$-thal after splenectomy did not require fewer red blood cell transfusions, indicating that not only splenic function contributes to the higher need for red blood cell transfusions. Although baseline hemoglobin levels do not differ between HbSS and HbS/$\beta^{0/+}$-thal, thalassemia trait may increase the need for red blood cell transfusion by aggravating anemia during acute complications. In contrast, patients with HbS/β^{++}-thal and HbSC required very few or no transfusions, confirming that acute anemia and VOC triggering transfusion are rare in these genotypes.[25,40]

The participation of many centers in the registry allowed for the analysis of patients with rare genotypes such as those with HbS/β^+-thal. However, the small number of patients still limits statistical

analyses and calls for validation in other populations. It must be borne in mind that despite several plausibility controls of data entry into the national German SCD registry there is no formal source data verification. Therefore, laboratory parameters and data on clinical complications are likely less complete than in clinical trials. In addition, treatment of SCD and accuracy of documentation in the registry may vary between treatment centers and distort results by a center effect. Nevertheless, our data demonstrate that comparing the clinical course of patients with SCD requires thorough genetic classification and correction for environmental factors. Treatment with hydroxyurea is one such environmental factor which is more frequent in the German SCD registry patients compared to patients from the US,[41] France,[42] Belgium,[43] and Spain.[44] Additionally, the effects of further genetic modifiers on the diverse phenotypic aspects of SCD should be analyzed by genome-wide association studies. However, such studies require large numbers of clinically well-annotated samples that can only be collected by international collaborations.

The marked genetic heterogeneity of patients in the national German SCD registry enabled for a direct comparison of hematologic and clinical phenotypes between different genotypes of SCD. This comparison confirmed the expected milder phenotype of HbS/β^{++}-thal and HbSC. However, it also revealed the surprising finding that patients with HbS/β-thal who express less than 14% HbA are at a higher risk of experiencing acute splenic sequestration and requiring red blood cell transfusion, even when compared to HbSS patients.

AUTHOR CONTRIBUTIONS

PA, LT, and JBK collected and analyzed data, designed the research, and wrote the manuscript. VW and AK-S performed statistical analyses. HC, SL, RG, MB, LO, DH, AJ, AEK, and JBK established the SCD registry and designed the research. JBK and AEK designed and supervised the study.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The protocol of the SCD registry is available in German language at www.sichelzellkrankheit.info. For original data, please contact joachim.kunz@med.uni-heidelberg.de. As the informed consent does not allow disclosure of individual patients' data, only aggregated data can be provided upon request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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