Received: 8 August 2024 | Accepted: 23 November 2024

DOI: 10.1111/bjh.19941

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

SHORT REPORT

Red Cells and Iron

Iron overload in hereditary spherocytosis: Are genetic factors the cause?

Lucie Donaty[1] | **Muriel Giansily-Blaizot**[1,2] | **Ivan Bertchansky**[2] | **Séverine Cunat**[1] | **Vincent Azoury**[1] | **Perrine Mahe**[2] | **Patricia Aguilar Martinez**[1,2]

[1]Department of Biological Haematology, Montpellier University Hospital, Montpellier, France

[2]Reference Centre for Rare Red Blood Cell Disorders, Montpellier University Hospital, Montpellier, France

Correspondence
Patricia Aguilar Martinez and Lucie Donaty, Department of Biological Haematology, Montpellier University Hospital, Montpellier, France.
Email: p-martinez@chu-montpellier.fr and l-donaty@chu-montpellier.fr

Funding information
European Reference Network on Rare Haematological Diseases, Grant/Award Number: 10108571; European Union

Summary
Non-transfusional iron overload (IOL) in hereditary spherocytosis (HS) is poorly documented compared with other red blood cell disorders. We studied 13 HS adults with confirmed IOL to identify potential genetic factors. Using a next-generation sequencing panel of 46 genes related to HS, anaemia and iron metabolism, we found no association between IOL and the genes involved in HS nor the *HFE*:p.(Cys282Tyr) variant responsible for hereditary haemochromatosis. However, potential genetic factors contributing to IOL were identified in some patients, including variants in *HJV* (haemojuvelin), *SLC40A1* (ferroportin), *PKLR* (pyruvate kinase), *ABCG5 and ABCB8*, highlighting the need for larger studies.

KEYWORDS
genetic modifiers, hereditary spherocytosis, hyperferritinaemia, iron overload

INTRODUCTION

Hereditary spherocytosis (HS) is the most common inherited red blood cell (RBC) disorder leading to chronic haemolysis in Northern Europe. It is caused by variants in genes encoding key proteins of the RBC membrane cytoskeleton: ankyrin (*ANK1*), band 3 (*SLC4A1*), β-spectrin (*SPTB*), α-spectrin (*SPTA1*) and protein 4.2 (*EPB42*). Clinical manifestations ranged from compensated to severe chronic haemolysis with complications including anaemia and gallstones.

Iron overload (IOL) is common in hereditary stomatocytosis, another RBC membrane disorder, but is poorly documented in HS. Some HS patients develop IOL without transfusions, and the cause remains unclear.

The link between HS and genetic IOL has been demonstrated since the 1950s, primarily in HS patients with hereditary haemochromatosis (HH). While initial reports focused on homozygous haemochromatosis,[1,2] recent cases suggest that heterozygous forms can also result in IOL in the absence of any chronic transfusions.[3,4] However, some recent reports failed to find *HFE* C282Y as a cause of IOL in HS patients.[5]

This study aims to investigate genetic factors that may contribute to IOL in HS patients.

PATIENTS AND METHODS

From a cohort of 140 patients diagnosed with HS at Montpellier University Hospital (France) between 1996 and 2022, we selected patients with chronic hyperferritinaemia (ferritin levels >200 μg/L in females and >300 μg/L in males) confirmed on several occasions (at least 3 months after the initial measurement). Demographic, clinical, biological, genetic and therapeutic follow-up data were collected (Tables 1 and 2). The cohort was registered as collection DC-2008-417, at the Centre for Biological Resources (CRB) of CHU Montpellier, France, and all patients who underwent genetic testing gave written informed consent.

Next-generation sequencing (NGS) was performed on an Illumina MiSeq® instrument. The panel included 46 genes associated with hereditary haemolytic anaemias, RBC

TABLE 1 Characteristics of the 13 patients with hereditary spherocytosis and proven iron overload included in this study. Grey shades represent abnormal value, abnormal clinical condition or therapeutic intervention.

Patient ID	Gender	Age at diagnosis (years)	Hgb (g/dL)	MCV (fL)	Direct bilirubin (μmol/L)	LDH (UI/L)	Haptoglobin (g/L)	Reticulocytes (G/L)	Splenomegaly	Splenectomy	Gallstone	Transfusion (*n*=)	Phlebotomy	Iron chelation	Family history of HS	Other
1	F	10	12.0	92	24	206	<0.1	544	Y	N	Y	1	N	N	N	Family iron overload
2	F	2	12.1	98	83	206	<0.1	566	Y	N	Y	0	N	N	NA	
3	M	27	13.1	91	41	213	1.22	339	Y	Y	N	0	N	N	N	
4	M	44	15.2	106	5	NA	<0.1	170	N	N	N	0	Y	Y	N	
5	M	22	14.4	91	29	210	<0.1	332	Y	N	Y	0	N	N	NA	Bone marrow reconversion of the knees
6	M	24	12.9	100	44	435	<0.1	380	N	N	N	0	Y	N	Y	
7	M	4	12.1	102	72	NA	<0.1	395	Y	N	N	1	N	N	NA	
8	M	41	13.3	97	22	214	<0.1	191	N	N	Y	0	Y	N	Y	
9	M	24	12.8	94	28	310	<0.1	359	Y	N	Y	1	Y	N	NA	
10	F	11	12.4	84	52	296	<0.1	498	Y	N	N	0	N	N	Y	Bone marrow reconversion of the knees
11	M	69	15.0	93	33	262	<0.1	267	N	N	N	0	Y	N	NA	
12	F	69	9.5	93	11	224	<0.1	323	N	N	Y	1	N	Y	Y	
13	F	NA	9.8	88	23	NA	<0.1	330	Y	N	Y	2	N	N	Y	
	Sex ratio = 0.6	Mean = 29	Mean = 12.7	Mean = 95	Mean = 36	Mean = 258		Mean = 361	Y = 8/13 (62%)	Y = 1/13 (8%)	Y = 7/13 (54%)	Y = 5/13 (38%)	Y = 5/13 (38%)	Y = 2/13 (15%)		

Note: Biological data were collected outside the haemolytic crisis and before any treatment (splenectomy or treatment of IOL), except for patient #12 for whom data were available after the start of iron chelation therapy. Spherocytes were observed in varying numbers (ranging from 1% to 20%) in the blood film of all patients, and three patients also had 'mushroom red cells' (#1, #3, #9).

Abbreviations: F, female; Hgb, haemoglobin level; HS, hereditary spherocytosis; M, male; MCV, mean corpuscular volume; N, no; NA, not available; Y, yes.

TABLE 2 Red cell and iron phenotypes and genotypes of the studied patients with hereditary spherocytosis and iron overload. Grey shades represent abnormal value, abnormal clinical condition or therapeutic intervention.

	Red blood cell parameters								Iron parameters					
	Genetic of spherocytosis												Genetic	
Patient ID	Gene	Variant (htz)	ACMG class	α^{lely}	Osmotic resistance	Pink test	EMA test	Ektacyto-metry	S. Ferritin (μg/L)	TSat (%)	LIC (μmol/g)	Age at MRI	Variant (htz)	Other variant (htz)
2	*ANK1* NM_020476.3	Deletion (exon 5–7)		Htz	+	+	+	+	315	22	23	34		
10		c.5097-33G>A	4	Hmz	NA	+	+	NA	413	30	42	59		
13		c.4918C>T p.(Gln1640Ter)	5	Wt	+	+	+	NA	639	36	60	75		
1		c.3701delC p.(Thr1234Metfs*15)	4	Htz	+	+	+	+	453	67	69	41		
4		c.2981C>T p.(Pro994Leu)*	4	Htz	−	−	−	+ (atypical spherocytosis)	809	50	90	50	NM_213653.4 *HJV*:c.863G>A p.(Arg288Gln) (class 3)	
7		c.2170C>T p.(Gln724Ter)	5	Htz	+	+	+	NA	2452	72	99	39		
3	*SPTA1* NM_003126.4	c.4610A>T p.(Lys1537Ile)	3	Hmz	+	+	+	+	307	43	37	27		
9		c.1677+1G>C	5	Htz	NA	+	−	+	557	41	210	48		NM_007188.5 *ABCB8*: c.931G>A p.(Ala311Thr) (class 3)
5	*SPTB* NM_001024858.3	c.4010delA p.(Lys1337Serfs*3)	5	Htz	NA	+	+	+	351	44	45	23	NM_014585.5 *SLC40A1*:c.744G>T p.(Gln248His) (class 3)	NM_138555.3 *KIF23*:c.1823G>A p.(Arg608His) (class 3)
6		c.4885C>T p.(Gln1629Ter)	4	Htz	+	+	+	NA	547	44	170	25		NM_022436.3 *ABCG5*:c.1762+1G>T (class 4)
12		c.5474_5486del p.(Gly1825Alafs*71)	4	Wt	+	+	+	NA	860	NA	230	69		
8	*SLC4A1* NM_000342.4	c.2510C>T p.(Thr837Met)	5	Htz	NA	+	+	+	872	28	100	41		NM_000298.6 *PKLR:* c.1516G>A p.(Val506Ile) (class 3)
11		c.1800G>C p.(Lys600Asn)	4	Htz	+	+	+	+	1334	48	131	68		
Mean±SD									762±585	44±15	100±67	46±17		

Note: Eleven of 13 patients had positive pink and EMA tests and two patients required ektacytometry to confirm the diagnosis. LIC was classified according to Henninger et al.[14]: one patient (#2) had a normal LIC (<32 μmol/g), five patients (#3, #10, #5, #13, #1) had borderline IOL (36≤LIC<75 μmol/g), two patients (#4, #7) had mild IOL (75≤LIC<100 μmol/g), two patients (#8, #11) had moderate IOL (100≤LIC<150 μmol/g) and three patients (#6, #9, #12) had moderate to severe IOL (150≤LIC<300 μmol/g). The Mobidetail annotation platform including the Mobidic Prioritization Algorithm (MPA) score was used to classify the variants according the ACMG classification. [ACMG class 3: Variant of Uncertain Significance (VUS); ACMG class 4: Likely pathogenic; ACMG class 5: Pathogenic].

Abbreviations: −, normal test; +, abnormal test; hmz, homozygous; htz, heterozygous; LIC, liver iron concentration; NA, not available; WT, wild type; wt, wild type.

*For this *ANK1* missense variant, we performed family segregation, which supports the pathogenicity of the variant (one sister, with a mild HS phenotype, carried the same variant, whereas the second asymptomatic sister did not).

enzymopathies and rare IOL conditions (Table S1). Genetic variants were classified according to the ACMG rules.

RESULTS

Chronic hyperferritinaemia was absent in the 99 individuals under 18 years of age, whereas it was found in 22 of the 44 adults. Only two of these exhibited secondary hyperferritinaemia (one patient with congestive hepatopathy, the other with multiple transfusions). Of the remaining 20 patients, we focused on the 13 who benefited from genetic analysis for diagnosis purposes (Table S1). They had a variable number of haemolytic crises. Only one patient had undergone splenectomy while five had received episodic transfusions. Anaemia was present in only two patients, but all had elevated reticulocyte counts. Most had spherocytes on blood films. The diagnosis of HS was confirmed by phenotypic testing in all 13 patients (Table 2).

The mean ferritin level was 762 µg/L [307–2452], with only four patients exhibiting elevated transferrin saturation (TSat). Liver MRI showed IOL in 12 out of the 13 patients, ranging from borderline to moderate–severe (Table 2). We found no significant correlation between LIC and ferritin levels or TSat, nor between ferritin levels and reticulocytes or haemoglobin levels. Six patients were offered therapeutic interventions, including phlebotomy and/or iron chelation (Table 1).

Of the 13 patients, 11 carried heterozygous causative variants in genes associated with HS: six in *ANK1*, two in *SLC4A1* and three in *SPTB*. One patient was a compound heterozygote for an *SPTA1* variant and the αLELY allele. The last carried an *SPTA1* missense variant of uncertain significance (VUS) associated with the homozygous αLELY allele (Table 2).

None of the 13 patients carried the common *HFE*: p.(Cys282Tyr) variant, responsible for HH. However, two potentially pathogenic variants were identified in other genes associated with rare non-HFE haemochromatosis. One patient had a variant in *HJV*, associated with juvenile haemochromatosis, and another carried an *SLC40A1* variant implicated in Bantu siderosis ('African haemochromatosis'). No potentially pathogenic variants were found in the *HFE*, *HAMP, TFR2* or *FTL* genes.

In addition, some patients carried rare variants in genes linked to other RBC disorders or IOL, namely, *PKLR*, encoding pyruvate kinase (PK), a key enzyme in RBC metabolism, *ABCG5* implicated in sitosterolaemia and *ABCB8*, encoding a mitochondrial iron transporter (Tables, 1,2).

DISCUSSION

In an attempt to better understand why IOL manifests in some HS patients, we conducted a retrospective study of HS patients with chronic hyperferritinaemia. We studied 13 well-characterized individuals who underwent LIC measurement by liver MRI and NGS sequencing.

None of these patients had received chronic transfusions, a major cause of acquired IOL in patients with chronic haemolytic anaemia, nor did they have chronic inflammation or liver steatosis. This prompted us to investigate their genetic background, specifically focusing on genes involved in RBC disorders and iron metabolism. This is the study with the largest number of HS patients with IOL to date. In a previous study of five patients, no genetic abnormalities were found.[6]

Our first step was to confirm the diagnosis of HS at the molecular level. Recent studies have shown that NGS analysis can help correct misdiagnosed cases of rare anaemias.[7] This is essential to distinguish HS from other RBC disorders such as hereditary stomatocytosis or PK deficiency. Indeed, both of them are associated with hyperferritinaemia (HF) or IOL.

The molecular diagnosis of HS was unambiguous in 11 patients. Of note, patient #4 had a missense *ANK1* variant for which family segregation studies support the pathogenicity. The remaining two patients (#3 and #9) had complex genotypes, with an *SPTA1* variant occurring *in trans* with the αLELY allele. This allele is linked to elliptocytosis when combined with a pathogenic *SPTA1* variant located in the N-terminal part of the protein. Recent studies suggest that when the αLELY allele is associated *in trans* with a pathogenic *SPTA1* variant encoding the remainder of the protein, it may slightly worsen the phenotype by increasing reticulocyte counts and decreasing haemoglobin levels.[8]

We subsequently investigated whether some of the molecular variants responsible for HS might be associated with IOL. We found no overrepresentation of any specific HS gene abnormalities in patients with hyperferritinaemia or IOL, although larger studies are needed to confirm this. However, we did identify additional genetic factors in four of the seven patients with more severe IOL and in one young patient with elevated ferritin but normal LIC.

We next examined genes involved in iron metabolism. None of the 13 patients carried the *HFE*: p.(Cys282Tyr) variant, the most frequently reported genetic cause of IOL in HS patients. However, we identified two potentially pathogenic variants in genes associated with rare forms of IOL in three patients. Patient #4, with an LIC of 90 µmol/g, carried the *HJV*:p.(Arg288Gln) variant. A single heterozygous variant of the HJV gene, implicated in juvenile haemochromatosis, cannot fully explain IOL. Nevertheless, several authors consider that heterozygous variants in this gene, in combination with other genetic abnormalities, may contribute to hereditary IOL through a digenic inheritance pattern. Patient #4 had no other known pathogenic variant.

Patient #5 did not have significant hepatic IOL, possibly due to his young age (23 years). He was diagnosed with HS at the age of 19 after bone marrow reconversion was found in his knees, a site not previously described in HS. He carried the *SLC40A1*:p.(Gln248His) variant associated with Bantu

siderosis.[9] This condition was once linked to consumption of iron-rich beer, but has now been confirmed to have a genetic basis. The variant is common in African and African-American populations, and studies show that heterozygous carriers, mainly men, have higher serum ferritin levels.

Patient #9, who had one of the highest LICs (210 µmol/g), carried the p.(Ala311Thr) variant in *ABCB8*. This gene encodes a transporter involved in iron export on the inner mitochondrial membrane. While loss-of-function mutations in mice lead to iron accumulation,[10] no pathogenic variants have been reported in humans.

We also identified associations with other variants in genes related to RBC disorders, where IOL is commonly observed. The *PKLR*:p.(Val506Ile) variant was found in patient #8, for whom no enzyme assays were available. This variant has previously been reported in a heterozygous state with a second *PKLR* variant in a patient with PK deficiency.[11] PK deficiency was not thought to aggravate the clinical phenotype of HS, except when associated with band 3 deficiency, as this may worsen the phenotype by exacerbating ATP deficiency.[12] And indeed, patient #8 had a molecular abnormality of the *SLC4A1* gene encoding protein band 3 and had a significant IO (LIC = 100 µmol/g), without further explanation.

Patient #6 had the *ABCG5*:c.1762 + 1G > T variant, located at the same nucleotide as a known pathogenic variant in sitosterolaemia,[13] an autosomal recessive disorder characterized by haemolysis and liver dysfunction. To our knowledge, there is no reported case of an HS patient carrying a pathogenic heterozygous *ABCG5* variant. Whether the relatively elevated LIC of this patient (170 µmol/g) may be related to this variant remains to be established.

Based on this study, we propose a diagnostic flowchart for HS patients who present with chronic hyperferritinaemia (Table S1, Figure S2).

CONCLUSION

These preliminary findings appear to support our initial hypothesis that genetic modifiers, whether in genes involved in RBC or iron disorders, may contribute to IOL development in certain HS patients and potentially explain the phenotypic variability observed between patients. However, further confirmation by analysis of a larger number of patients is required.

AUTHOR CONTRIBUTIONS

IB, PM and PAM examined the patients, VA and LD collected the phenotypic and genotypic data; MGB, SC and LD analysed the genetic variants, LD, MGB and PAM analysed the data and wrote the paper.

ACKNOWLEDGEMENTS

The authors thank Robert Navarro for referring some patients and Corinne Favier, Vanessa Debant, Fabienne Danton, for technical assistance.

FUNDING INFORMATION

This project is carried out within the framework of the European Reference Network on Rare Haematological Diseases (ERN-EuroBloodNet)—Project ID No 10108571. ERN-EuroBloodNet is partly co-funded by the European Union under the framework of the Fourth EU Health Programme.

This work benefited from the expertise of the Centre of Biological Resources (CRB) of the CHU de Montpellier.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Yes.

PATIENT CONSENT STATEMENT

Yes.

ORCID

Lucie Donaty https://orcid.org/0009-0000-6751-2383
Patricia Aguilar Martinez https://orcid.org/0000-0003-4311-2945

REFERENCES

1. Edwards CQ, Skolnick MH, Dadone MM, Kushner JP. Iron overload in hereditary spherocytosis: association with HLA-linked hemochromatosis. Am J Hematol. 1982;13(2):101–9.
2. Brandenberg JB, Demarmels Biasiutti F, Lutz HU, Wuillemin WA. Hereditary spherocytosis and hemochromatosis. Ann Hematol. 2002;81(4):202–9.
3. Mohler DN, Wheby MS. Hemochromatosis heterozygotes may have significant iron overload when they also have hereditary spherocytosis. Am J Med Sci. 1986;292(5):320–4.
4. Fargion S, Cappellini MD, Piperno A, Panajotopoulos N, Ronchi G, Fiorelli G. Association of hereditary spherocytosis and idiopathic hemochromatosis. A synergistic effect in determining iron overload. Am J Clin Pathol. 1986;86(5):645–9.
5. Ichiche M, Lacor P, Hoorens A, Vanden Brande J, Brussaard H, Vanstraelen D. Congenital spherocytosis with hereditary hemochromatosis without pathogenic mutations in the HFE gene. Eur J Intern Med. 2004;15(7):460–2.
6. Tole S, Amid A, Baker J, Kuo K, Pugi J, Carcao M. Mild hereditary spherocytosis without accompanying hereditary haemochromatosis: an unrecognised cause of iron overload. Acta Haematol. 2019;141(4):256–60.
7. Bianchi P, Fermo E, Vercellati C, Marcello AP, Porretti L, Cortelezzi A, et al. Diagnostic power of laboratory tests for hereditary spherocytosis: a comparison study in 150 patients grouped according to molecular and clinical characteristics. Haematologica. 2012;97(4):516–23.
8. Svidnicki MCCM, Zanetta GK, Congrains-Castillo A, Costa FF, Saad STO. Targeted next-generation sequencing identified novel mutations associated with hereditary anemias in Brazil. Ann Hematol. 2020;99(5):955–62.
9. Rivers CA, Barton JC, Gordeuk VR, Acton RT, Speechley MR, Snively BM, et al. Association of ferroportin Q248H polymorphism with elevated levels of serum ferritin in African-Americans in the hemochromatosis and iron overload screening (HEIRS) study. Blood Cells Mol Dis. 2007;38(3):247.
10. Ichikawa Y, Bayeva M, Ghanefar M, Potini V, Sun L, Mutharasan RK, et al. Disruption of ATP-binding cassette B8 in mice leads to cardiomyopathy through a decrease in mitochondrial iron export. Proc Natl Acad Sci USA. 2012;109(11):4152–7.

11. Zarza R, Moscardó M, Alvarez R, García J, Morey M, Pujades A, et al. Co-existence of hereditary spherocytosis and a new red cell pyruvate kinase variant: PK mallorca. Haematologica. 2000; 85(3):227–32.
12. van Zwieten R, van Oirschot BA, Veldthuis M, Dobbe JG, Streekstra GJ, van Solinge WW, et al. Partial pyruvate kinase deficiency aggravates the phenotypic expression of band 3 deficiency in a family with hereditary spherocytosis. Am J Hematol. 2015; 90(3):E35–E39.
13. Lam CW, Cheng AWF, Tong SF, Chan YW. Novel donor splice site mutation of ABCG5 gene in Sitosterolemia. Mol Genet Metab. 2002;75(2):178–80.
14. Henninger B, Alustiza J, Garbowski M, Gandon Y. Practical guide to quantification of hepatic iron with MRI. Eur Radiol. 2020;30(1):383–93.

SUPPORTING INFORMATION

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

How to cite this article: Donaty L, Giansily-Blaizot M, Bertchansky I, Cunat S, Azoury V, Mahe P, et al. Iron overload in hereditary spherocytosis: Are genetic factors the cause? Br J Haematol. 2026;208(2):691–696. https://doi.org/10.1111/bjh.19941