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Characterization of ferroportin disease and SLC40A1-related hemochromatosis – Results from the EASL non-HFE registry

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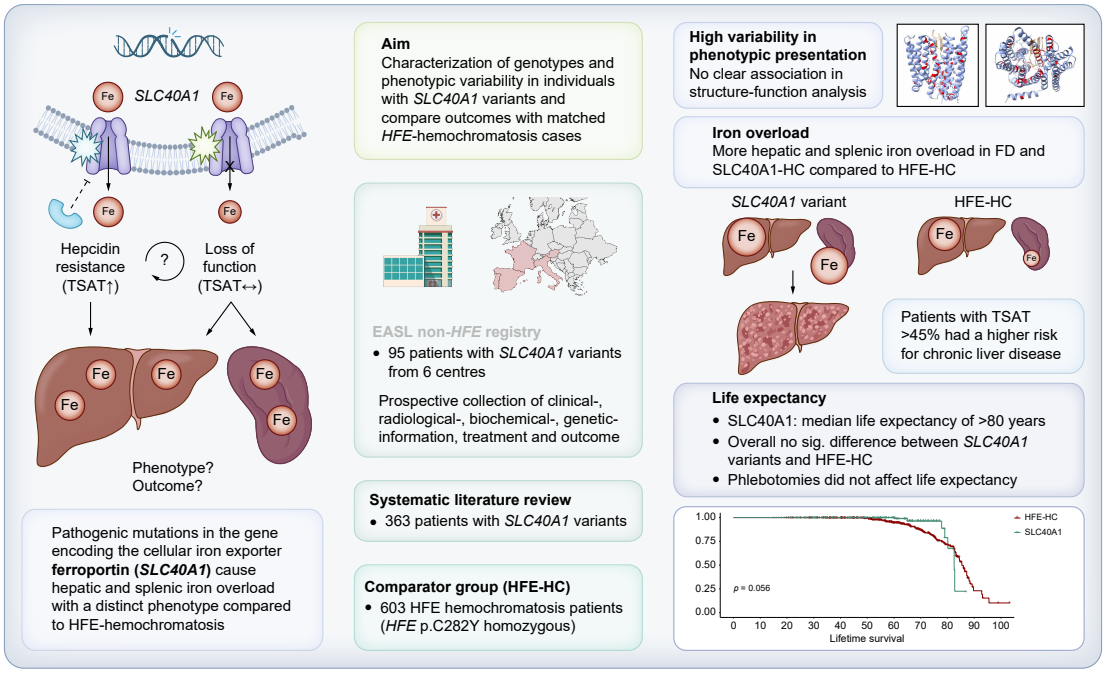
Graphical abstract



Highlights

- Comprehensive characterization of *SLC40A1*-related iron overload disorders.
- *SLC40A1* variants show high variability in genotype-to-phenotype correlation.
- The presence of spleen iron overload is characteristic of ferroportin disease.
- Risk for liver disease is higher in SLC40A1-related hemochromatosis compared to ferroportin disease.
- Lifetime survival was not significantly affected by phlebotomy treatment.

Impact and implications

Clinical management of individuals with *SLC40A1* variants has largely been extrapolated from HFE-related hemochromatosis despite fundamental pathophysiological differences. Our study provides detailed phenotypic characterization that supports diagnosis and distinction of these rare iron overload disorders. Long-term follow-up shows preserved life expectancy, unaffected by phlebotomy, underscoring the need to critically assess phlebotomy on an individualized basis. Patients with SLC40A1-related hemochromatosis (transferrin saturation >45%) had a higher prevalence of chronic liver disease than those with ferroportin disease, suggesting that elevated transferrin saturation and hepatic iron drive disease progression, which can guide risk stratification and clinical decision making.

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Characterization of ferroportin disease and SLC40A1-related hemochromatosis – Results from the EASL non-HFE registry

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Background & Aims: Pathogenic variants in the cellular iron exporter ferroportin (*SLC40A1*) cause hepatic and splenic iron overload. Low to normal transferrin saturation (TSAT) and iron accumulation in Kupffer cells with high splenic iron distinguish ferroportin disease (FD) from *SLC40A1*-related hemochromatosis (SLC40A1-HC), which are both caused by variants in *SLC40A1*. The aim of our study was to describe pathogenic mutations in *SLC40A1*, phenotypic variability in affected patients and compare outcomes with *HFE*-related hemochromatosis (HFE-HC).

Methods: The international EASL non-*HFE* hemochromatosis patient registry prospectively collected clinical, radiological, biochemical, and genetic data for 95 patients with *SLC40A1* variants from six centers. Additionally, 363 patients were identified by a systematic literature review. As a comparator, 603 patients diagnosed with HFE-HC were included.

Results: The FD phenotype presented in 65.5% affected individuals. Patients with FD were younger at diagnosis and more often female than those with SLC40A1-HC. *SLC40A1* variants were associated with higher hepatic and splenic iron concentrations compared to the HFE-HC group. Variability in phenotypic presentation was high among patients with *SLC40A1* variants, and a genotype-to-phenotype correlation could only explain a small proportion of this variation. Variants that directly affect the metal binding site in ferroportin more likely presented with high TSAT. Patients with the SLC40A1-HC phenotype (TSAT >45%) had a higher risk of fibrosis. Life expectancy was similar between patients with *SLC40A1* variants and matched patients with HFE-HC. Most individuals with *SLC40A1* variants (73.2%) received regular phlebotomies, which were not associated with differences in life expectancy.

Conclusions: Mutations in *SLC40A1* cause a highly variable disease spectrum with hepatic and splenic iron overload. Fibrosis risk is higher in patients with elevated TSAT.

Clinical Trial number: Not applicable.

Introduction

After *HFE* in hemochromatosis, *SLC40A1* is the second most commonly affected gene in patients with hepatic iron overload without anemia.[1,2] *SLC40A1* encodes human ferroportin, a transmembrane protein of 571 amino acid residues, which is the only known iron efflux transporter in humans and mediates iron absorption and recycling.[3] Its iron transport activity is controlled by the hormone hepcidin, which regulates serum iron by inactivating ferroportin before inducing its internalization and degradation.[4,5] Alterations either in the iron transport activity of ferroportin or in its interaction with hepcidin can cause iron overload.[6–8]

The hallmarks of *HFE*-related hemochromatosis (HFE-HC) and of pathogenic *SLC40A1* variants are high serum ferritin and elevated hepatic iron concentrations.[9,10] In contrast to HFE-HC, where affected patients almost invariably present with high transferrin saturation (TSAT) and low spleen iron,[11] patients with *SLC40A1* variants have highly variable TSAT and spleen iron concentrations.[12–16] These variations have been attributed to a genotype-to-phenotype correlation, with two

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distinct phenotypes in patients with *SCL40A1* variants.[17–20] Genetic variants inhibiting the iron export function of ferroportin cause ferroportin disease (FD) with low TSAT and high spleen iron due to iron overload in macrophages.[21] In contrast, patients with low spleen iron, high TSAT and iron primarily in hepatocytes, have mutations in *SLC40A1* that preserve the iron export function, but render ferroportin resistant to hepcidin-mediated inactivation.[22] In 2022, an updated definition of hemochromatosis was proposed by the BIOIRON society, including a new nomenclature for patients with *SLC40A1* variants.[23] FD, which was formerly called type 4A or classical ferroportin disease is characterized by high ferritin, low to normal TSAT and reticuloendothelial iron overload, affecting Kupffer cells in the liver and the spleen macrophages. The phenotype is caused by loss-of-iron-export-function variants.[16,24] Distinct from this phenotype are patients with *SLC40A1* mutations causing hepcidin resistance. This disease is now classified as *SLC40A1*-related hemochromatosis (SLC40A1-HC) and was formerly known as hemochromatosis type 4, FD type B, or non-classical FD.[23] This phenotype is characterized by high TSAT and ferritin levels, and iron overload in hepatocytes, sparing macrophages, Kupffer cells and the spleen.[16,18]

More recent reports challenge this concept by showing that certain ferroportin variants can impair iron export function and alter hepcidin binding.[19,20] Recently, the published structure of hepcidin-bound ferroportin provided new insights into iron homeostasis and revealed that ferroportin molecules need to be loaded with metal for hepcidin binding.[5] Studies on the ferroportin structure have also unraveled the iron export function at atomic resolution and identified those residues that are critical for iron export.[25]

From a clinical perspective, this classification is also of limited value because some patients present with ambiguous phenotypes and it is not known if the two distinct phenotypes are associated with different risks of developing progressive liver fibrosis. The unknown long-term effects of *SLC40A1* variants and the highly variable disease presentation also pose challenges for disease management. Especially, potentially beneficial effects of phlebotomy are mainly inferred from HFE-HC and have never been demonstrated in clinical studies that included patients with *SLC40A1* variants. In contrast to patients with HFE-HC, patients with FD have been reported to show lower tolerance to phlebotomy and are more likely to become anemic, but rarely have progressive fibrosis or cirrhosis.[8,16]

The aim of our study was to describe the phenotype and assess the impact of phlebotomy in a large cohort of patients with *SLC40A1* variants by combining real-world data from a longitudinal follow-up of previously published patients and from the prospective EASL non-HFE registry.

Materials and methods

EASL non-HFE registry

The EASL (European Association for the Study of the Liver) sponsored and European Iron Club endorsed non-*HFE* registry was established in 2016 to enable multicenter collection of patient data on FD as well as other rare iron loading disorders. Registry data was collected both retrospectively as well as prospectively with informed consent, using the electronic case report form "Askimed".[26] This study was approved by the local ethics committee of the Medical University of Innsbruck (protocol number: AN2016-0096 362/4) as well as by the local ethics committees responsible for the contributing centers. Data entry started in 2017 and final data retrieval was in July 2023. Six centers provided 103 case entries; eight entries had to be excluded due to missing data and 95 cases could be included for further analyses (Fig. 1).

Systematic literature review and collection of longitudinal data from published patients

In addition to the prospective registry, a systematic literature search was performed on the 15th of June 2024, using both Pubmed as well as Web of Science (Fig. S1) with the following search strategy: a combination of #1 ('SLC40A1' or 'ferroportin' or 'ferroportin1' or 'IREG' or 'IREG1' or 'SLC11A3' or 'FPN' or 'FPN1' or 'HFE4' or 'MTP1') and #2 ('mutation' or 'variant'). No study filter was applied. The search generated 598 results in Pubmed between 1989 and June 2024 and 776 results in Web of Science between 1993 and June 2024. The titles and abstracts of each result were screened by two independent investigators (MRT and BS) and, if there was a conflicting assessment, a third inverstigator (HZ) was consulted. After the exclusion of 472 double and 798 other entries (review articles, non-human studies, conference papers, unsuitable clinical studies, *i.e.* studies without any phenotypic information), data from 104 publications were included. Finally, all corresponding authors were contacted and we received additional data as well as follow-up data for 71 cases. From the systematic review 363 additional patients could be included.

Ferroportin disease and SLC40A1-related hemochromatosis

Individuals with *SLC40A1* variants with reported TSAT (n = 391) were classified either as "ferroportin disease" (FD) when TSAT was ≤45% or as "*SLC40A1*-related hemochromatosis" (SLC40A1-HC) when TSAT was >45%.

HFE C282Y homozygous comparator group

HFE p.C282Y homozygous patients were included as a comparator group in our study.

All patients were genotyped in Innsbruck between 1997 and 2023 (as previously described[27]) and matched 1:1 to the *SLC40A1* cohort using exact matching based on sex and age (allowing a maximum difference of 3 years) using the R package "MatchIt".[28] Since not all parameters were available for each individual in the *SLC40A1* cohort, separate sub-analysis sets were created for each parameter, also using 1:1 exact matching based on sex and age. Subsequently, patients with FD and SLC40A1-HC were individually matched to *HFE* p.C282Y homozygous patients for separate comparisons.

Amino acid sequence and 3D-structure of ferroportin

For the sequence and the exact positions of the transmembrane domains, the entry for human ferroportin Q9NP59 in the UniProt database was used (https://www.uniprot.org). Chimera X was employed to show all variants mapped on the

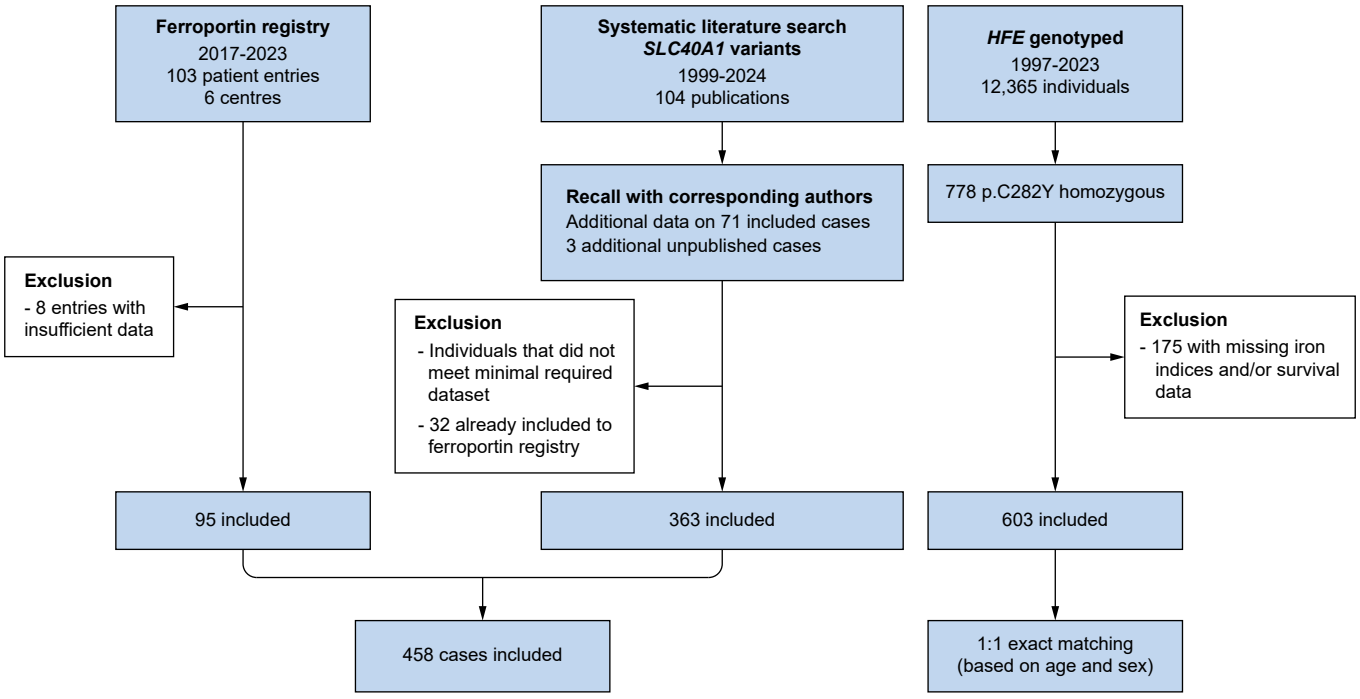


Fig. 1. Consort diagram. 458 cases with *SLC40A1* variants were included in our study (95 from the non-HFE registry, 363 from the literature). Exactly matched (based on sex and age with up to 3 years of tolerance) patients with HFE-related hemochromatosis served as a comparator group.

published 3D structure of ferroportin,[5] made available by the RCSB PDB (protein database), entry 6WBV. The monoclonal antibody Fab45D8 is not shown in our figure. R Studio v 4.3.3[29] and the package "bio3d" were used to calculate the distance between the cobalt atom (where the iron atom would reside under physiological conditions) and the C-alpha atom of each residue.[30]

Statistics and graphs

Quantitative data are presented as mean ± standard deviation for normally distributed variables and as median with first and third quartiles for non-normally distributed variables. Normality was assessed using the Kolmogorov-Smirnov test. Frequencies are reported as absolute numbers or percentages as indicated. Quantitative variables were compared using unpaired Student's *t* test or the non-parametric Mann-Whitney *U* test as appropriate. Contingency tables were tested for significance using the Fisher exact or $\chi 2$ tests. Lifetime survival was calculated from date of birth to date of death or last follow-up and graphed using the Kaplan-Meier method, and groups were compared with the log-rank test, using R packages "survival", "survminer", "ggpubr", "dplyr" and "ggsci".[31–35]

For principal component analyses (PCA), ferritin, age, TSAT, serum iron, sex, position, the relative distance of the C-alpha atom of each variant and the presence within a transmembrane domain were included as covariates. R packages "FactoMineR" and "factoextra" were used for PCA.[36,37] Data were analyzed using IBM Statistics SPSS v27.0, graphs were created using GraphPad Prism,[38] R v.4.3.3 with the package "ggplot2",[29,39] CorelDRAW,[40] Biorender and ChimeraX.[41] Alpha was set at 0.05 for statistical significance and all tests were conducted using two-sided *p* values.

Results

Characteristics of patients with *SLC40A1* variants

We included 458 individuals with *SLC40A1* variants in our study (Fig. 1). Table 1 shows a comparison of patient demographics, serum iron parameters, liver and spleen iron concentrations and liver-related outcomes between patients with FD and SLC40A1-HC. TSAT was reported for 391 individuals. Two thirds of patients (65.5%) presented with FD (TSAT ≤45%). This group was significantly younger, had lower serum iron, lower ferritin and a lower median liver iron concentration compared to the SLC40A1-HC (TSAT >45%) group. Phlebotomy was performed more frequently in SLC40A1-HC than in FD (88.2% *vs.* 65.7%, p <0.001) and patients with SLC40A1-HC more often had chronic liver disease (steatosis, fibrosis and cirrhosis) than those with FD (Table 1). Hepatocellular carcinoma was diagnosed in two male patients, one in the FD and one in the SLC40A1-HC group. Their respective ages at presentation of iron overload were 74 and 45 years. For the younger patient with FD-related HCC, steatosis was the only significant comorbidity reported (Table 1), while the patient with SLC40A1-HC had occult HBV infection.

Sex was reported for 421 patients, of whom 176 (41.8%) were females. Men presented with significantly higher serum ferritin and TSAT compared to women. Males were also more likely to have been treated with phlebotomy (Table 2).

Variants and function

We conducted a genotype-phenotype analysis to investigate the cause of high variability in disease presentation. In total, 79 variants in *SLC40A1* were reported affecting 58 residues. The most common variant was V162del. An overview of all 79

Characterization of *SLC40A1* variants

Table 1. Patient demographics, iron parameters, treatment and liver-related outcomes of 391 individuals with available transferrin saturation measurements.

	n	FD (n = 256)	n	SLC40A1-HC (n = 135)	*p* values
Female % (n)	235	46.4% (109)	127	33.1% (42)	0.009-
Age at diagnosis (years)	242	41.5 (±18.1)	126	47.3 (±17.4)	0.004*
Serum iron (μg/dl)	140	85 (70–117)	65	201 (150–247)	<0.001°
Ferritin (μg/L)	256	1,563 (±2,035)	131	3,645 (±3,639)	<0.001*
Transferrin (mg/dl)	102	242 (212–266)	23	216 (190–233)	0.003°
Transferrin saturation (%)	256	28 (22–35)	135	81 (62–92)	-
Liver iron concentration (μmol/g)	64	100 (54–200)	26	222 (184–328)	<0.001°
Spleen iron concentration (Hz)	9	187 (±153)	0	-	-
Phlebotomy treatment % (n)	137	65.7% (90)	68	88.2% (60)	<0.001-
Steatosis (n)	42	26.2% (11)	14	78.6% (11)	<0.001-
No fibrosis (n)	58	84.5% (49)	24	29.2% (7)	<0.001-
Fibrosis F1–F3 (n)		12.1% (7)		45.8% (11)	
Compensated cirrhosis (n)		3.4% (2)		25.0% (6)	
Decompensated cirrhosis (n)		0% (0)		0% (0)	
Hepatocellular carcinoma (n)	66	1.5% (1)	13	7.7% (1)	0.304-

Data are presented as percent (number), mean (SD) or median (first and third quartile), *p* values were calculated using Student's *t* test (*), Mann-Whitney *U* test (°) or $\chi 2$ test (-) as indicated.

Table 2. Sex differences among individuals with *SLC40A1* variants (based on 421 individuals with available sex data).

	n	Males (n = 245)	n	Females (n = 176)	*p* values
Age at diagnosis (years)	238	45.0 (32.0–58.0)	166	41.4 (26–55.1)	0.127°
Serum iron (μg/dl)	108	130 (±75)	99	113 (±54)	0.071*
Ferritin (μg/L)	236	2,714 (±2,968)	173	1,602 (±1,920)	<0.001*
Transferrin (mg/dl)	65	230 (210–258)	65	232 (208–266)	0.486°
Transferrin saturation (%)	210	50 (±28)	151	40 (±23)	<0.001*
Liver iron concentration (μmol/g)	50	163 (±105)	46	147 (±115)	0.475*
Phlebotomy treatment % (n)	123	84.6% (104)	98	64.3% (63)	<0.001-
Steatosis (n)	37	51.4% (19)	27	22.2% (6)	0.022-
No fibrosis (n)	49	61.2% (30)	43	81.4% (35)	0.167-
Fibrosis F1–F3 (n)		26.5% (13)		11.6% (5)	
Compensated cirrhosis (n)		10.2% (5)		7.0% (3)	
Decompensated cirrhosis (n)		2.0% (1)		0% (0)	
Hepatocellular carcinoma (n)	44	4.5% (2)	46	0% (0)	0.236-

Data are presented as percent (number), mean (SD) or median (first and third quartile), *p* values were calculated using Student's *t* test (*), Mann-Whitney *U* test (°) or χ^2 test (-) as indicated.

reported *SLC40A1* variants is shown in Fig. 2A and the supplementary material. Forty-four of 58 affected residues (75.9%) are localized in transmembrane domains, making up for 81.0% of all reported variants in our cohort (93.7% of all individuals with *SLC40A1* variants).

To reduce inter-individual variability, the genotype-to-phenotype correlation was conducted only for variants with data reported in >3 patients. Ten variants were exclusively associated with either low or high TSAT. Of these, p.D157N, p.W158C, p.D181N, p.S209L, p.G468S and p.R489K were solely associated with the FD phenotype (TSAT <45%). In contrast, all patients with p.Y64N, p.C326Y, p.Y333H and p.H507R presented with the SLC40A1-HC phenotype (TSAT ≥45%). In the remaining variants, both phenotypes were observed (Fig. S2).

Next, we investigated if the position of the affected residue within the 3D structure of ferroportin determines TSAT. To achieve this aim, we mapped the variants onto the published 3D structure of ferroportin (Fig. 2B).[5] From structural data, the relative distance of the C-alpha atom of each mutated residue to the central iron atom in ferroportin, which is also the hepcidin binding site, was calculated. Then a correlation analysis was conducted between the calculated distance and the reported TSAT values for each variant. This analysis showed a significant negative association, which supports a structure-function relationship, where variants in close proximity to the hepcidin binding site are more likely to cause the SLC40A1-HC phenotype (Fig. S3), possibly due to hepcidin resistance.

To determine to what extent the variability in phenotypic presentation could be explained by patient demographics, genetics, and clinical-biochemical parameters, a PCA was performed. Ferritin clusters with age and sex, but less so with TSAT, which is closely related with serum iron. Instead, the structural distance of the affected residue from the central iron atom/hepcidin-binding site clustered with TSAT. The weak association between these parameters suggests that the phenotypic variability in ferritin and TSAT is only partially explained by patient demographic and genotype-phenotype relationships (Fig. 3).

Disease phenotype of *SLC40A1* variants and *HFE* hemochromatosis

To compare the disease phenotype and prognosis of FD and SLC40A1-HC with those of HFE-HC, we analyzed serum iron parameters, liver and spleen iron concentrations, and survival against a cohort of 603 *HFE* C282Y homozygous patients. To account for differences in age at presentation and sex distribution, the *SLC40A1* cohort was age and sex-matched with the HFE-HC cohort (Fig. 1). Individuals in the *SLC40A1* cohort

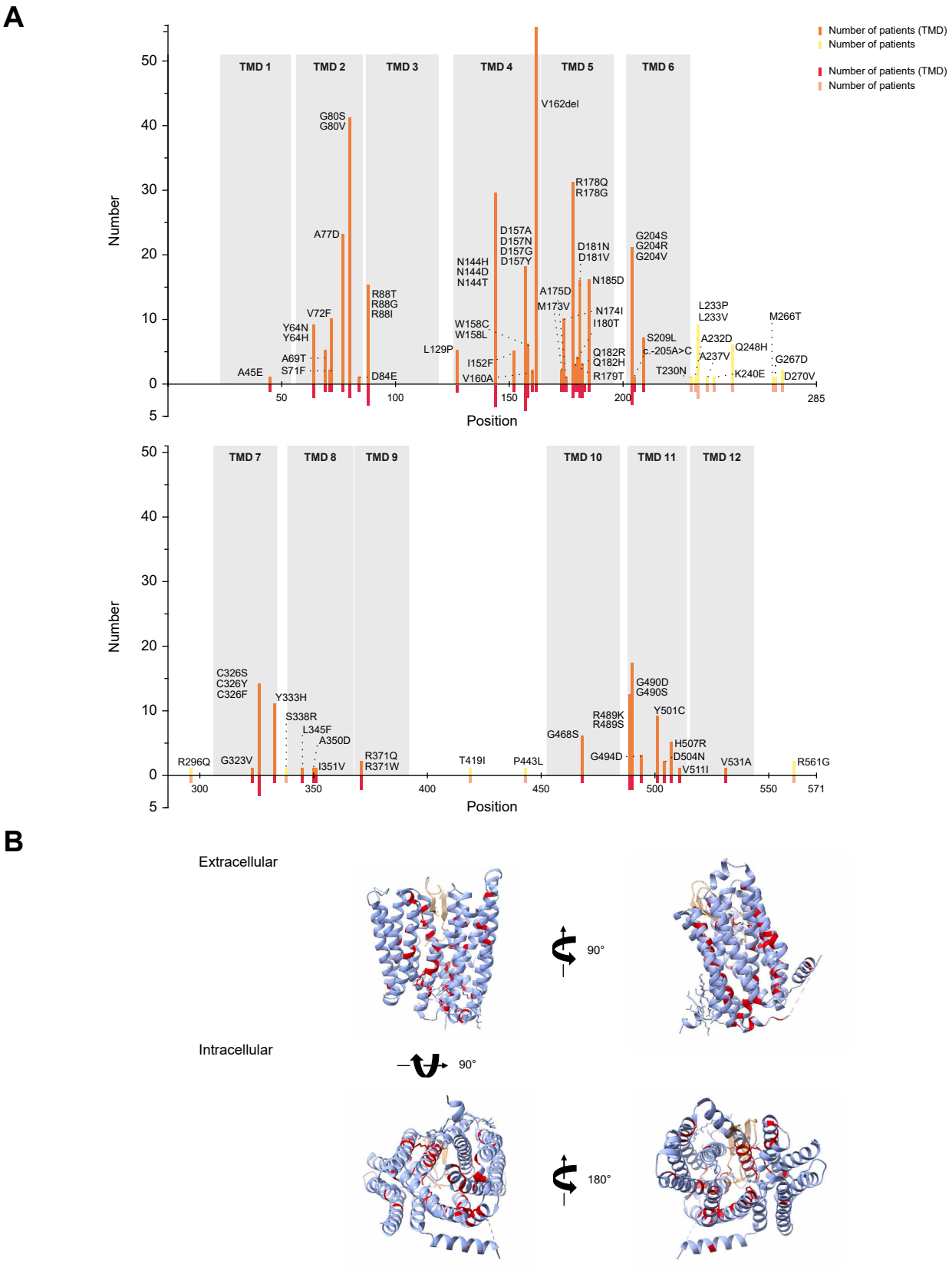


Fig. 2. Reported *SLC40A1* variants in the primary sequence and 3D structure of ferroportin with reported variants highlighted in red. (A) Seventy-nine different mutations were found; 44 of 58 affected residues (75.9%) are localized on transmembrane domains, accounting for 81.0% of the variants in our cohort (64 of 79) and 93.7% of all patients (429 of 458). The most common variant was V162del, affecting 12% of the cohort (55 patients). (B) Hepcidin is shown as a beige structure in the center. All variants are listed with additional information in the supplementary material.

Characterization of *SLC40A1* variants

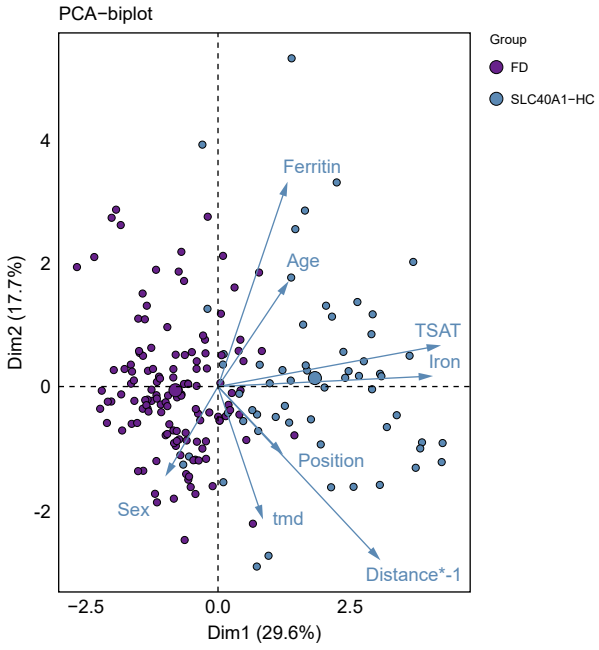


Fig. 3. Biplot of the PCA. Variability in demographic parameters, serum iron parameters and their association with location of mutant residue in the primary sequence (position), as well as their distance from the central iron atom in the 3D structure of ferroportin (distance), were analyzed by dimension reduction analysis. Each dot represents a patient included in the analysis and the blue arrows show the Eigenvector of the indicated parameter. Light blue dots are patients with FD whereas patients with SLC40A1-HC are shown in dark purple. The figure highlights that the phenotypic variability in ferritin and TSAT can only in part be explained by demographic variables and genotype-to-phenotype correlation. FD, ferroportin disease; PCA, principal component analysis; SLC40A1-HC, SLC40A1-related hemochromatosis; TSAT, transferrin saturation.

presented with significantly higher median ferritin and transferrin concentrations, whereas the HFE-HC group had significantly higher serum iron and TSAT (Table 3 and S1 and S2). Comparison of liver and spleen iron concentrations showed significantly higher median liver and spleen iron concentrations in the *SLC40A1* variant cohort when compared to patients with HFE-HC (Fig. 4). No difference in pancreatic iron was found.

Life expectancy in patients with *SLC40A1* variants

Patients with *SLC40A1* variants and 1:1 matched patients with HFE-HC had a high median life expectancy of >80 years of age with no significant difference between groups (Fig. 5A). In subgroup analyses, longer life expectancy was observed in the FD group, but not in the SLC40A1-HC group, compared to the matched patients with HFE-HC (Fig. S6A,B). Phlebotomy treatment was not associated with differences in life expectancy among individuals with *SLC40A1* variants (Fig. 5B), either overall or after stratification by phenotype into FD or SLC40A1-HC (Fig. S6C,D). Life expectancy did not differ significantly between FD and SLC40A1-HC (Fig. 5C), nor was it affected by the presence of hepatic iron overload (Fig. 5D).

Discussion

The present study describes the phenotypic presentation and the spectrum of genetic variants reported in the largest cohort to date of patients with variants in *SLC40A1*. With 458 patients included, *SLC40A1* variants represent the commonest genetic cause of primary hepatic iron overload after HFE-HC. In contrast to *HFE* p.C282Y, which has an allele frequency of 0.06–0.12 in individuals of North-Western European ancestry, pathogenic *SLC40A1* variants have been reported globally, and the frequency of pathogenic alleles was calculated at 0.0004.[27,42,43] We show that in contrast to HFE-HC, on average, patients with *SLC40A1* variants have more severe hepatic iron overload, more splenic iron, higher serum ferritin and transferrin. The matched study design prohibits direct comparison of age at presentation and sex distribution between FD and HFE-HC or SLC40A1-HC and HFE-HC. Comparison with previously published, unselected HFE-HC cohorts suggests that patients with FD present at a younger age and are more likely to be female than patients with HFE-HC. In contrast, patients with SLC40A1-HC are typically older and more frequently male compared to those with HFE-HC.[27]

A unifying feature among patients with this SLC40A1-HC phenotype – defined as a TSAT >45% – was that variants affected residues that likely interact with hepcidin.[5,23] These variants could thereby cause impaired hepcidin function and result in a phenotype similar to that of HFE-HC, where iron overload is caused by inappropriately low circulating hepcidin.[44] Low hepcidin in HFE-HC further causes low spleen iron concentrations.[11] In the present study, spleen iron measurements were only available in a minority of patients with FD. This parameter could be obtained from abdominal R2*-MRI sequences and could add additional diagnostic information.[45] Qualitative assessment of MR images has previously shown that spleen iron was apparently low in selected cases with *SLC40A1* variants that affect hepcidin binding.[19,46,47] In contrast, the majority of the remaining variants were associated with significant variation in TSAT. Only a few residues (p.D157N, p.W158C, p.D181N, p.S209L, p.G468S and p.R489K) were exclusively associated with the FD phenotype – defined by TSAT <45%. A recent study identified p.D157 and p.R489 as key residues for iron binding at the inner gate of ferroportin,

Table 3. Comparison of individuals with *SLC40A1* variants and HFE-HC after 1:1 exact matching based on age and sex.

	n	*SLC40A1* variant (n = 358)	n	*HFE*-HC (n = 358)	*p* values
Female % (n)	358	39.7% (142)	358	39.7% (142)	-
Age at diagnosis (years)	358	46.5 (±15.8)	358	44.2 (±15.5)	-
Serum iron (μg/dl)	187	107 (76–160)	187	195 (153–226)	<0.001°
Ferritin (μg/L)	352	1,515 (749–3,102)	352	493 (217–992)	<0.001°
Transferrin (mg/dl)	124	238 (±45)	124	199 (±39)	<0.001*
Transferrin saturation (%)	317	36 (26–67)	317	77 (59–86)	<0.001°
Hemoglobin (g/dl)	86	14.2 (±1.3)	86	14.4 (±1.6)	0.369*

Data are presented as percent (number), mean (SD) or median (first and third quartile), *p* values were calculated using Student's *t* test (*) or Mann-Whitney *U* test (°) as indicated. HFE-HC, *HFE*-related hemochromatosis.

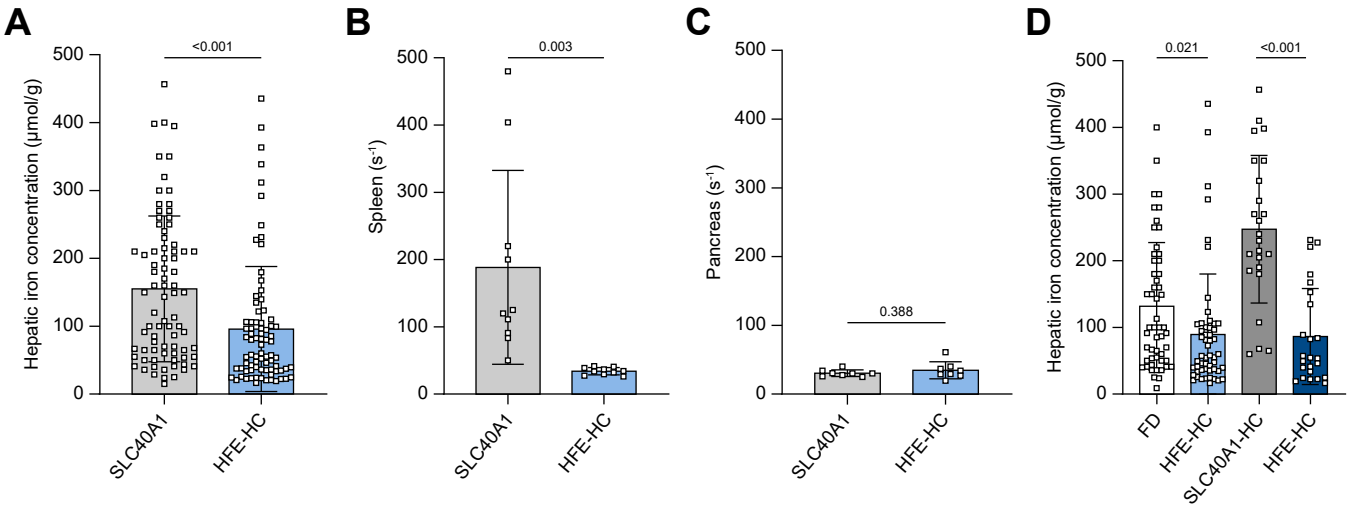


Fig. 4. Comparison of hepatic, splenic and pancreatic iron concentrations in individuals with *SLC40A1* variants and the exactly matched cohort of HFE-HC patients. (A) Individuals with *SLC40A1* variants present with significantly higher hepatic iron concentrations than *HFE* C282Y homozygous patients (p <0.001), (D) both with the FD and SLC40A1-HC phenotpye (p = 0.021 and p <0.001, respectively). (B) Splenic iron is also significantly higher in the FD cohort (p = 0.003), whereas (C) no significant difference in pancreatic iron concentrations was observed (p = 0.388). The scatter dot plots show means with SD, p values were calculated using an unpaired Student's t test. FD, ferroportin disease; HFE-HC, HFE-related hemochromatosis; SLC40A1-HC, SLC40A1-related hemochromatosis.

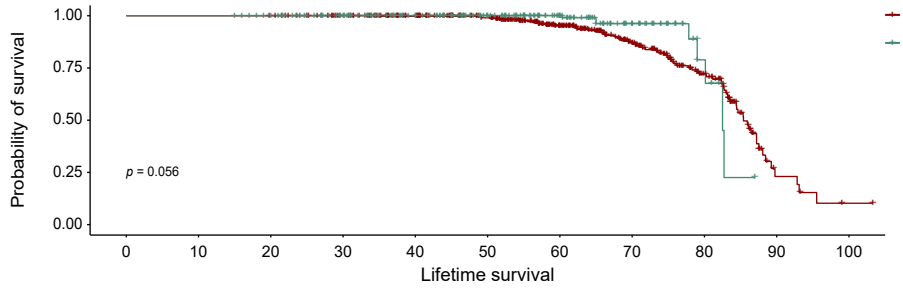
which provides a mechanistic explanation for this finding.[25] The fact that many *SLC40A1* variants have only been reported in a few patients limits the validity of assigning a phenotype – FD or SLC40A1-HC – to these rarer, private variants. Originally, the FD phenotype was attributed to subcellular mislocalization of mutant ferroportin or degradation of the mutant protein,[22,48] but more recent studies have shown that some ferroportin variants have impaired iron export function despite correct localization at the plasma membrane.[49] These findings led to the concept that active iron export function by ferroportin is required for hepcidin binding,[19,50] which has been confirmed in later studies on the hepcidin-ferroportin complex.[5] Recent studies showed the dual loss of iron export function and hepcidin binding, which was found to result in an FD phenotype.[20] The present study shows that the majority of ferroportin variants cannot be associated with a specific phenotype. The principal component- and regression analysis supports this concept because older age was associated with higher TSAT, especially in males. However, age explained only 7% of the variability in TSAT in females and 3% in males (Fig. S5). The finding could also indicate that TSAT is of limited value in phenotypic classification of *SCL40A1* variants. Furthermore, the present study shows that both sexes were more equally affected in patients with FD, whereas in HFE-HC, the male-to-female ratio is typically 3.5 to 1.[51] Taken together, these findings show that the type of *SLC40A1* variant, age, and sex only partly explain the variability in phenotypic presentation. This also raises the question of whether TSAT alone is a reliable indicator of the phenotype in FD and what the ideal threshold would be to distinguish FD from SLC40A1-HC. The biologic variability in TSAT is very high,[52] which represents a major limitation when using this parameter alone for the phenotypic classification of patients with *SLC40A1* variants as FD or SLC40A1-HC.

MRI can be considered a complementary tool to TSAT, because MRI can be used to non-invasively detect splenic iron overload, which is suggestive of FD. Hyperferritinemia is a common finding in clinical practice and is often attributable to metabolic conditions without significant iron overload, where ferritin is a marker of advanced fibrosis.[53] In contrast, *SLC40A1*-associated disorders are rare and characterized by hepatic and/or splenic iron overload. Therefore, further evaluation with MRI and genetic testing should be reserved for individuals with a strong clinical suspicion of parenchymal iron overload.[9,54]

The clinical relevance for the distinction between SLC40A1-HC from FD is based on the concept that the risk for fibrosis progression would be higher in SLC40A1-HC.[55,56] The present study supports this concept with a higher prevalence of fibrosis, cirrhosis and steatosis in SLC40A1-HC than in FD. This difference was not associated with changes in life expectancy. Comparison of cirrhosis risk with the HFE-HC group is limited by the low number of events in each subgroup. Furthermore, life expectancy was not different between patients with *SLC40A1* variants and age- and sex-matched individuals with HFE-HC, where median overall survival from birth was >80 years in both groups. Subgroup analyses suggested a trend toward longer life expectancy in the FD group compared to matched patients with HFE-HC. No differences in life expectancy were found when patients were stratified by phlebotomy treatment. This analysis indicates that iron removal may not always be associated with improved outcome. Whether specific subgroups of patients benefit from therapeutic venesection – and how these individuals should be identified – remains an open question. Addressing this will require prospective, controlled studies, which may also help define treatment thresholds and targets that are appropriate for SLC40A1-HC, and potentially distinct from those established for HFE-HC. Likewise, the utility of emerging hepcidin-directed therapies in the treatment of FD or SLC40A1-HC is currently unknown and could be investigated in future studies.[12]

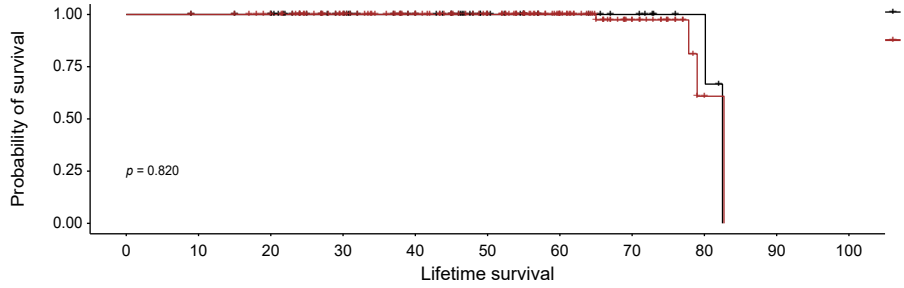
Characterization of *SLC40A1* variants

A



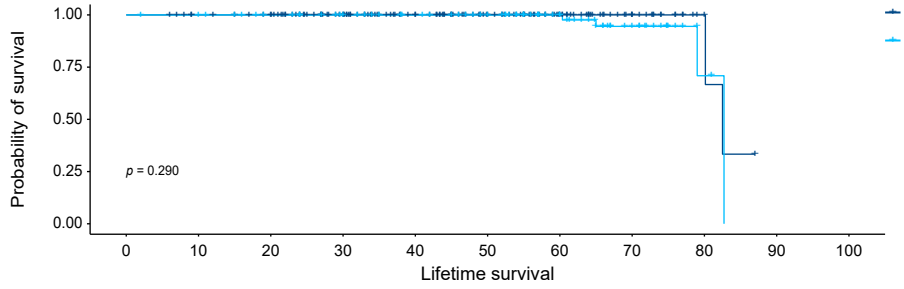
Number at risk	HFE-HC	366	366	365	350	322	284	202	127	64	6	1
	SLC40A1	366	366	354	308	243	179	107	45	7	0	0
Cumulative number of events	HFE-HC	0	0	0	0	1	3	13	27	44	69	72
	SLC40A1	0	0	0	0	0	0	0	3	5	8	8

B



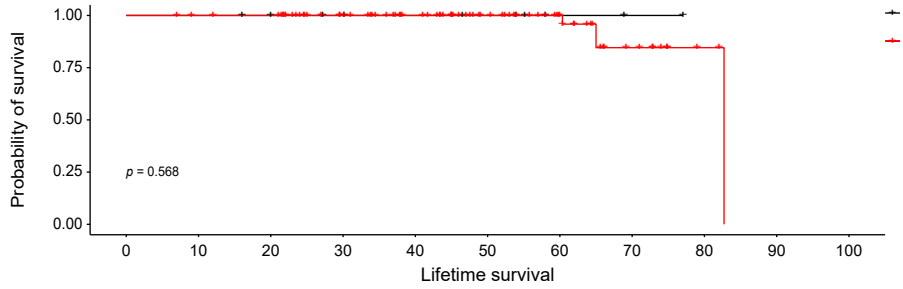
Number at risk	No phlebotomy	60	59	58	44	35	21	12	9	3	0	0
	Phlebotomy	173	172	167	147	118	98	57	24	2	0	0
Cumulative number of events	No phlebotomy	0	0	0	0	0	0	0	0	0	2	2
	Phlebotomy	0	0	0	0	0	0	0	1	3	4	4

C



Number at risk	FD	242	235	223	179	135	95	54	24	4	0	0
	SLC40A1-HC	126	125	114	108	94	74	46	19	3	0	0
Cumulative number of events	FD	0	0	0	0	0	0	0	0	0	2	2
	SLC40A1-HC	0	0	0	0	0	0	0	2	3	4	4

D



Number at risk	No hepatic iron overload	10	10	9	7	6	5	2	1	0	0	0
	Hepatic iron overload	100	98	97	82	62	41	26	9	2	0	0
Cumulative number of events	No hepatic iron overload	0	0	0	0	0	0	0	0	0	0	0
	Hepatic iron overload	0	0	0	0	0	0	0	3	3	4	4

Fig. 5. Comparison of survival. (A) There was no significant difference in life expectancy between individuals with *SLC40A1* variants and the HFE-HC group (p = 0.056). (B) Phlebotomy was not associated with differences in life expectancy in patients with *SLC40A1* variants (p = 0.820), neither was (C) TSAT at a cut-off of 45% (*i.e.* classifying the patients as either FD or SLC40A1-HC, p = 0.290), nor (D) the presence or absence of hepatic iron overload (p = 0.568). *P* values were determined using the log-rank test. FD, ferroportin disease; HFE-HC, HFE-related hemochromatosis; SLC40A1-HC, SLC40A1-related hemochromatosis.

In conclusion, variants in *SLC40A1* are the second most common genetic cause of hepatic iron overload. In contrast to the most common cause, HFE-HC, the phenotypic presentation of patients with *SLC40A1* variants is highly variable, and this variability can only partly be explained by a genotype-to-phenotype correlation. The finding that patients with a TSAT >45% have a higher cirrhosis prevalence suggests that high TSAT and high liver iron drive disease progression, but the optimal threshold for distinguishing between FD and SLC40A1-HC might be higher.

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Abbreviations

FD, ferroportin disease; HC, hemochromatosis; HFE-HC, HFE-related hemochromatosis; PCA, principal component analysis; SLC40A1-HC, SLC40A1-related hemochromatosis; TSAT, transferrin saturation.

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Conflict of interest

The authors declare no conflicts of interest pertaining to this manuscript.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

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Data availability

All data can be made available by contacting the corresponding author and after obtaining approval for data sharing by the non-HFE registry consortium.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2025.10.016.

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Author names in bold designate shared co-first authorship

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